



A PROCEDURE FOR DECEREBRATING THE RAT BY THE ANEMIA METHOD

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Recently, we have undertaken to extend our study of the early development of reflex behavior to the albino rat. One of the first difficulties encountered was the elimination of the effects that an anesthetic may have on the fetuses. It is essential to know that the movements and reflexes observed in embryos and early fetuses are not depressed and obscured by ether used to render the pregnant animal unconscious. Experience in studying cats has led us to believe that the use of any anesthetic at the time observations are being made upon the fetuses is detrimental to the condition of the latter. In order to avoid the use of drugs during observations, our animals are first decerebrated, preferably by the anemia method of Pollock and Davis '24).

Swenson ('28, '29, '31 b) and Angulo ('30 a and b, '32 a and b) have been engaged in studying the fetal movements of the rat for a number of years. They recognize the necessity of eliminating the use of ether. The former (Swenson, '31 a) has experimented with a method for rendering the rat unconscious by means of increased intracranial pressure. Angulo has used Swenson's ('25) original method for producing cerebral hyperemia (and, presumably, the unconscious state) by ligating the common carotid arteries and the jugular veins.

At first, we employed the latter method in our work on the albino rat, but have discarded it for the following reason: It does not produce a state of complete unconsciousness and,

¹ Contribution no. 189.

consequently, ether, in quantities that seem to be relatively large for a small animal,² must be administered while the fetuses are being studied. In fact, after tying carotid and jugular vessels and allowing a short time for recovery from ether, we have observed relatively little deviation from normal behavior of the rat and, in some cases, entirely normal reactions within a day. At best, Swenson's method produces a partial passive hyperemia of the head—not a complete cerebral anemia. To eliminate any question of the effect of drugs, we have developed a procedure for rendering the rat entirely unconscious and motionless even when the anesthetic is removed. Our method is, essentially, a modification of that of Pollock and Davis ('24), who developed the technique of producing the decerebrate state in the cat by ligating the carotid and basilar arteries. One cannot easily reach the basilar artery from the mouth of the rat; therefore we have used a cervical, trans-pharyngeal approach.

THE METHOD

After the rat has been etherized, it is fastened to an operating board, back down and nose extended and fixed. The thorax and abdomen are blanketed with cotton batting to keep the animal warm. A skin incision is begun about $\frac{3}{4}$ inch behind the chin and carried along the median plane for about $1\frac{1}{2}$ inches. The submaxillary glands are freed by blunt dissection, retracted forward, and kept out of the field with mosquito artery forceps. The sternomastoid muscles are retracted laterad and the common carotid arteries are ligated. After this has been done, ether must be continued until the final stage of the operation is reached.

Next, the infrahyoid muscles are detached from the hyoid bone and larynx and are allowed to retract toward the thorax, displaying the larynx, trachea, and thyroid gland. The larynx-

² Regardless of the measured quantity of ether administered to a rat or to any other animal with the blood supply of the brain intact, the concentration in the blood is more or less constant for a given degree of anesthesia (Ronconi, '23). It is meaningless to record the amount poured on a mask during the operation, because this in no way indicates the blood concentration.

geal and thyroid vessels are usually secured with double ligatures between which they are sectioned. This should be done to prevent hemorrhage into the trachea in the next stage. The trachea and oesophagus are sectioned immediately below the larynx after the trachea has been cannulated with a glass tube of suitable size. The anesthetic is then applied by a sponge at the end of the cannula; care must be exercised to avoid condensation of the ether inside the glass. The larynx and pharynx are grasped by mosquito forceps and carried forward from the field. With these out of the way and with the trachea movable, it is relatively easy to make a careful dissection of the carotid arteries. All the remaining branches of these vessels are ligated to eliminate the possibility of collateral circulation to the brain. When this has been done, the prevertebral muscles are detached from the skull by means of a tiny, sharp-pointed strabismus hook (curvature with a diameter of 2 mm.); they retract caudad and are automatically removed from the field of operation. Thus, the basilar portion of the occipital bone is reached.

Here a 2-mm. opening is made in the skull with a motor-driven burr. The last tissue-paper-thin plate of bone is removed with the strabismus hook and a pair of delicate, blunt, ophthalmological dressing forceps, and the dura mater is torn away with the hook or a small iris knife (6-mm. blade). The basilar artery, often surprisingly large in proportion to the size of the animal, is thus displayed as it reaches the pons from the ventral surface of the medulla oblongata. In order to place a ligature about it, it is sometimes necessary to dissect it free from the brain. A fine needle or the small iris knife is used for this purpose. Some of the minute branches to the pyramids may be broken, but the hemorrhage from these is usually slight. An aneurysm needle was fashioned from the finest obtainable, smooth, straight needle by heating the eye-end in a flame and bending it to the proper shape. The pointed end was fastened into a handle. This instrument is used to carry a piece of fine thread (B & B no. 1 twisted surgical silk) under the basilar artery about 1 mm.

caudal to the pons. The loop of thread is grasped with fine forceps and the aneurysm needle is then withdrawn. It need scarcely be said that the tying of the knot must be effected with care; this is done with two pairs of these forceps.³

The anesthetic is discontinued just before the basilar artery is ligated. The larynx and salivary glands are replaced and the skin-flaps are brought together around the tracheal cannula by means of metal skin clips. The animal is removed from the operating board and given constant post-operative care for a half hour or more, during which all effects of the anesthetic disappear.

The duration of the operation need not exceed 25 or 30 minutes and the total loss of blood need not exceed 1 cc.; it is usually only a few drops. Disturbances of temperature regulation and respiration seem to be the most serious consequences of the operation. Perfection in technique and care in maintaining a good surgical anesthesia entirely eliminate the respiratory difficulties which are prone to occur while the basilar artery is being dissected and ligated. Mucus which accumulates in the tracheal cannula can be removed with a whip of cotton on a dental brooch. The body temperature may drop several degrees during the course of the operation unless measures are taken to prevent it. Blanketing the animal on the table serves to retard the loss, and the application of warm wrappings after the operation will restore a small loss to normal within a short time. Heat must be used with discretion, because it is possible to force the temperature above normal. We have experienced little difficulty in keeping successfully operated animals alive and in good physical condition during several hours of experimentation.

Blood stasis and, consequently, anoxemia develop in the entire brain rostral to a plane transecting the medulla oblongata about 1 mm. caudal to the pons. Methylene blue, injected into the left ventricle of the heart, colors the brain

³ The fact that the rat will bleed to death in surprisingly few minutes in most cases of rupture of the basilar artery suggests that this vessel alone is of sufficient size to keep the brain supplied with blood.

caudal to this plane, but is not found rostral to it. One need not fear that embryonic movements are depressed by an anesthetic half an hour or more after decerebration of the parent. On the other hand, although the maternal rat is irritable and sometimes shows markedly exaggerated reflexes while being fastened in position to view the embryos, it thereafter lies quietly; one need not fear that consciousness remains when all vessels are properly ligated.⁴ These conditions, we believe, recommend the present technical procedure for studies on normal, living embryos and fetuses of the rat.

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⁴If the operation has been performed without hemorrhage from the branches of the basilar artery, and if the brain tissue has not been harmed, typical extensor rigidity develops in neck, leg, and tail muscles, and the animal displays the familiar caricature of standing.

CONTRACTILITY OF THE CILIATED NECK IN THE NECTURUS KIDNEY

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Ellinger and Hirt ('29, p. 201) interpreted their observations as indicating that the third segment in the frog's renal tubule offers resistance to the continuous passage of fluid, which resistance must be overcome by accumulation of fluid in the second segment. They made no suggestion, however, as to whether this resistance may be varied by changes in the diameter of the segment due to an active contractility. It was reported last year (Lucas and White, '32) that examination of fresh sections taken from the kidney of *Necturus maculosus* revealed the ciliated neck in either a contracted or relaxed state. Because these observations were made on excised tissue, the existence of similar changes in the intact animal remained questionable until the recent careful study by Singer ('33) showed that such changes take place in the frog's kidney in situ. His proof of the existence of such contractions in the living animal subject to the minimum manipulative injury makes it desirable to present certain additional observations on excised tissue which are not readily obtained by in vivo methods.

Thin sections of the kidney cut parallel to the ventral surface were obtained with a sharp-edged revolving disc. Tubules suitable for study contained nephrostome, glomerulus, ciliated neck, and the beginning of the proximal convoluted tubule. Their identification and continuity were readily de-

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terminated by the addition of a colored solution such as trypan blue, which was swept into the system by the nephrostome cilia.

Examination of such preparations showed clearly the expanded ciliated neck and within it the vigorously active cilia moving with a combined flagellar and vibratile motion. Many of the ciliated necks, however, were contracted in the excised tissue. The contraction is accomplished by decreasing the diameter of the tube without changing its shape and since there is no flattening or buckling of the walls, a definite lumen remains.

The changes which occur in the transformation from the expanded to the contracted state have been followed under the microscope and some of the causes are indicated. The contraction in the excised tissue occurs more rapidly than expansion. The rapidity with which it contracts, the completeness of the reaction once started and its resistance to reopening suggest that the contraction of the ciliated neck is due to an inherent contractility of the cells which form its walls or of the underlying basement membrane or both. No formed elements having this specific function are visible in material fixed and stained by the commonly used laboratory methods.

When the ciliated neck is collapsed, the cilia lie extended in its narrow lumen. They are apparently closely packed together and no evidence of attempted vibratory movements in any portion of a quiescent cilium has been observed. This inhibition of movement may be due entirely to mechanical restraint, but in view of other examples of ciliary inhibition the various possible causes should be considered. Carter ('26, '28) has demonstrated that the cilia may be inhibited by the action of the nervous system in the Nudibranch veliger, and Lucas ('33) has recently shown that the cilia on the frog's palate are normally at rest unless stimulated either externally or internally. Therefore, the inhibition in the cilia of the neck is perhaps of nervous origin or the ciliated cells may not be autonomous in their activity and thus come to rest when no longer stimulated by the pressure of fluids.

The order of events from the contracted to the expanded state has been followed under certain conditions on the excised tissue. If the proximal convoluted tubule belonging to a contracted ciliated neck is compressed with a fine rod so that the free passage of fluid is interrupted, the pressure within the tubule increases, due to the action of the nephrostome cilia. It was shown by White ('29) that this pressure may equal 8 to 11 cm. of water. The nephrostome cilia in these experiments were powerful enough to create a pressure sufficient to force open the ciliated neck. This opening is progressive, beginning at the junction of ciliated neck and nephrostome. Its rate of progression presumably depends upon the rate of pressure increase and the degree of resistance to opening offered by the neck: it may occur in a few seconds or require more than a minute. Each portion as it expands attains its full limit of expansion and the transitional zone between the opened and contracted portions is relatively short. There is no partial relaxation of the constricted portions in advance of the wave of expansion, nor has a relaxation of the central portion been noted with the two ends constricted. The point of greatest resistance appears to be that part of the neck adjacent to the capsule. As soon as any portion of the tube is expanded the cilia in that region become fully active, but are quiescent in the remaining regions. When the neck or any proximal part of it is contracted it is noted, by using a colored fluid, that the fluid fails to filter past the quiescent cilia, even under a positive pressure.

Particles, as well as fluids, may be forced backward through the opened tube into the capsule if the pressure is sufficiently high to overcome the downward action of the cilia in the neck. That this may occur in the living animal, particularly with fluids, is recorded by Lucas and White ('32), who found that the primary cause in such cases is apparently a blockage of the tubule by desquamated tubular cells. When particles are carried into the capsule the action of the cilia in that portion of the capsule adjacent to the neck whirls them around in this region and tends to prevent their dispersion

through the capsular space and apparently also down again into the neck.

The absence of circulating blood and the resulting cessation of normal glomerular function in the excised tissue renders progressive dilatation of the neck from the glomerulus downward of rather infrequent occurrence but it has been observed in one instance and in that case the series of events was the same as in the upward passage of fluid already described. A blood corpuscle escaped from the capsule and entered the neck accidentally, there being no pressure in the capsule because there was no circulation through the glomerulus. The progressive dilatation of the neck was always at the same level as the moving blood cell. The part of the tube behind the corpuscle remained expanded.

When obstruction of the proximal tubule has caused the accumulated fluid to open the ciliated neck, removal of the obstruction, allowing the rapid discharge of fluid, is followed by contraction of the ciliated neck. The contraction is always rapid and the sequence of events can hardly be followed. It is uncertain whether the contraction of the neck wall takes place progressively or simultaneously throughout the segment between capsule and junction with nephrostome. The contraction is due apparently to contractility of the cells rather than to a passive collapse due to either a mere return to the resting state or to a negative pressure produced by the action of the cilia. The manner of reopening already described is evidence for this conclusion.

Singer ('33) has noted a contracted condition in the ciliated junction between proximal and distal tubules of the frog. Its effect is to prevent the continuous passage of fluid from the proximal region. The observations on excised tissue indicate that when the glomerular pressure is sufficiently low the neck region above the junction of the nephrostome may likewise be constricted. The physiological significance of this contractile mechanism as a means for regulating intratubular pressure and rate of fluid passage is at present largely a matter of conjecture.

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OBSERVATIONS ON THE LIPOIDS IN THE RENAL TUBULE OF THE CAT

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TWO PLATES (SIX FIGURES)

INTRODUCTION

The renal tubule of the cat differs from that of other mammals in that great numbers of relatively large lipoid droplets can be demonstrated within the walls of the tubule. These droplets are restricted, in the main, to the convoluted portions of the tubule.¹ Christianna Smith ('20) described the differences in position and amount of lipoid in the kidney of the cat, the dog, the rabbit, and the rat, and to some extent, the variations with age and obesity in the cat's kidney. She described two forms of lipoidal deposit in the cat as well as in the other animals studied; spherical droplets in the proximal and rod-shaped in the distal portions of the convoluted tubules. Some observations were made on kidneys from old cats which were possibly pathological and on one pair of grossly pathological kidneys from a kitten (and in one case on an animal with a pathological liver), although the nature of the lesions were not described, nor was it explained why these animals were kept in the series studied. In her descriptions she gives the length of the cats but not their weight. She was led to believe that in the cat the presence of lipoid droplets in the convoluted portions of the renal tubule, which are physiologically very active, indicates that the lipoids are concerned in renal function, and that the variations in the

¹In discussing the renal tubule in this paper, the terminology will be used which designates as the proximal convoluted tubule the portion next to the glomerulus.

form of the lipoidal deposit may indicate differences in function in the various portions of the renal tubule.

Hartley ('07, '09), in studies on the lipoids of the viscera, found that the fatty acids from the heart, liver, and kidney were more unsaturated than the fatty acids of the connective tissue and other fat depots of the body. He pointed out that the more easily oxidized fatty acids, i.e., the more unsaturated, are found in centers of high metabolic activity; that they are chemically more active and hence more available for immediate use by the organism. Hartley did not examine the cat. Mottram ('16), however, found that in this animal the fatty acids extracted from the kidney were much more saturated than the fatty acids of the other viscera, and that, in some cases, the iodine value was strikingly lower than that of the fatty acids extracted from the fat depots of the body. With respect to the other viscera of the cat, his findings were in agreement with Hartley's in other mammals. Mottram also made histological observations on the kidney of the cat and noted that there was a great deal of lipid demonstrable in its renal tubule, which was invariably found in the convoluted portions and rarely in the straight portions. In some cases he observed lipid globules in the lumina of the blood vessels (lipaemia), and in others in the lumina of the convoluted portions of the renal tubule which he indicated might represent a lipuria. He made no report on the cat's urine.

Because of the fact that there were extractable lipoids in the tissues of the body which cannot be demonstrated microscopically, the term demonstrable or stainable lipid will be used in this paper. It is known that in certain pathological conditions intracellular lipoids become microscopically demonstrable in many tissues and parenchymatous organs, although the total extractable lipid content does not appear to have been increased. This phase will not be discussed here because the present study deals with a normal condition in which large lipid droplets are constantly visible.

Although the striking amount of demonstrable lipid in the renal tubule of the cat has long been known, its presence has

not been explained and no special significance has been attributed to it. The unusual amount of lipid and its peculiarly constant position are highly suggestive and a priori, the lipoids in the renal tubule of the cat may have some functional significance. The fact pointed out by Mottram that the fatty acids of the lipoids of the cat's kidney differ from those found in the renal tubule of other mammals is also suggestive, especially since they exist there in a form not readily available for metabolic activity.

In studies on urine, experimenters have apparently avoided the cat consistently, possibly because the position of the urethra in the female makes catheterization, and hence quantitative studies, difficult. In this paper the presence of certain refractile bodies, presumably lipid in nature, consistently seen in the urine of adult cats, is reported. The presence of lipid droplets in urine was not found to be reported in the literature examined, except in pathological conditions in the human, namely, lipid nephrosis and chyluria.

Inasmuch as the cat is a common laboratory animal, the author feels justified in emphasizing the histology of its kidney with reference to the lipoids present in its renal tubule, especially since there seems to be evidence which indicates that the lipoids in the renal tubule may have a significant role in renal function in this animal.²

METHODS

Because of the fact that certain pathological conditions and certain drugs cause fatty changes in the viscera, it was important to be certain that such was not the case in the animals used for study. Inasmuch as the source of the animals used was not known, except that they were the common 'alley' cat

² A. Witt ('20) wrote a dissertation entitled "Vorkommen und Bedeutung von Fett in den Nieren der Katze." Because of the highly suggestive title, much effort was spent in trying to obtain a copy. No copy could be found in any of the available library services in this country. Up to the time this paper went to press neither the dissertation nor an abstract of it has been located, nor has any suggestion as to the nature of its contents been found in any work of reference.

brought in by animal collectors, they were all kept in the laboratory and fed on scrap meat for at least a week before experiments or examinations of kidneys were begun. All appeared to be in good health and none of the organs examined seemed to be pathological, either grossly or under the microscope. One animal had been used several days previously for an experiment on the excretion of codeine. None of the others were previously used for experimental work.

Twenty-six cats of various ages were used. Twelve were fully grown adults, two were kittens about half-grown (averaging about 1.5 kg. in weight), four kittens were 10 days old, five were removed from utero at term, and three foetuses about 7.5 cm. in length were removed from the gravid uterus. Two cats were pregnant, and the foetuses above described were removed from their uteri.

In order to eliminate the possibility that the drugs used to anaesthetize the animals might cause fatty changes in the viscera, a number of anaesthetics were employed. Sodium amytal, administered intraperitoneally, was used for most of the adult cats. One was anaesthetized with chloroform and one with ether. In one case the kidneys were removed under local (procain) anaesthesia. The kittens 10 days old were etherized and the kittens at term were bled. One adult was bled from the femoral artery after local anaesthesia (induced by painting locally with phenol in oil) and died several hours later.

The kidneys and livers were removed, fixed in 15 per cent neutral formalin, and sectioned with the freezing microtome (20 to 35 μ , although the kidneys of the foetuses 7.5 cm. long were cut at 50 μ). They were stained with alkaline-alcoholic scarlet red, according to the method of Herxheimer (Bell, '10), counterstained with Ehrlich's hematoxylin, cleared in glycerine, and mounted in glycerine jelly. Sections were also stained with hematoxylin and eosin and mounted in dammar.

One animal was starved for 14 days, receiving nothing but water during that period, and during which it lost 44 per cent of its body weight. Another was fed on milk, one-half pint

daily, for 8 days and another was fed 200 cc. of cream 3½ hours before the kidneys were removed. In two animals, after one kidney had been removed, the ureter leading from the other was ligated, and a diuresis was induced with caffeine for a period of 2 hours before the remaining kidney was removed. This procedure distended the renal tubule and made it possible to examine and identify the contents of the tubule with more assurance than in the normal tubule in which the walls are closely approximated. The kidneys of the other animals were examined without any experimental procedure in order to determine the normal variations.

In all but five animals, and the three fetuses 7.5 cm. long, the urine was aspirated from the bladder with a hypodermic syringe when the abdomen was open, and in addition, urine was milked by vigorously massaging the abdomen toward the pubes, from fourteen adult cats whose kidneys were not examined. The urine was examined under the microscope in the recent state and after the addition of a small amount of scarlet red solution. Three hundred cc. of collected urine was concentrated over a water-bath and extracted with alcoholic-ether and petroleum-ether, for the lipid contained in it.

OBSERVATIONS

In the kidneys of all the adult animals examined, and of the two half-grown kittens, without regard for weight or sex, the convoluted portions of the renal tubule, both proximal and distal, were found to be literally filled with large lipid droplets. There was a tendency for intracellular lipid globules to concentrate somewhat around the nuclei. The droplets varied in size, the largest being about the same size as an erythrocyte. The nuclei and cytoplasm appeared to be in perfect condition (fig. 1). In several cases, and more clearly in those in which the ureter had been ligated for several hours to distend the renal tubule, lipid droplets were distinct within the lumina of the tubule (figs. 5 and 6). It was this observation which suggested an examination of the urine. Although the convoluted portions of the tubules were filled

with lipid droplets, other portions of the tubule were made equally conspicuous by the complete absence of stainable lipid in the cells. The glomeruli and collecting tubules were free of stainable lipid and only occasionally extremely fine lipid granules were visible in the medullary (Henle's) loops. When the frozen sections were stained with hematoxylin and eosin and run through the alcohols, the lipid was dissolved out and the renal tubules appeared full of vacuoles. No vacuoles were observed in sections stained for lipid. The difference in form between the droplets in the proximal and distal convoluted portions of the tubule, reported by Miss Smith, was not observed.

The picture seen in the very young kittens and fetuses is strikingly different from that seen in the adult. In the kitten about half-grown the condition in the renal tubule is essentially the same as in the fully grown adult. In the fetuses 7.5 cm. long extremely fine lipid granules were seen in the tubule at about the junction of the cortex with the medulla. At this stage the renal tubule is not fully differentiated. In the kitten at term there was very little demonstrable lipid except for a few medium-sized droplets found at the junction of the proximal convoluted tubule and the descending limb of the medullary loop. In the kittens 10 days old the same condition was found, but here the distribution of lipid was a little more extensive (fig. 2). Relatively large droplets, about the size of an erythrocyte, were found at the junction of the proximal convoluted tubule and the loop of Henle, but proximal to this, very fine lipid granules were seen extending for a short distance toward the glomerulus. The periphery of the cortex was entirely free of stainable lipid.

In the pregnant cats, from which the fetuses 7.5 cm. long and the kittens at term were removed, the kidneys showed a striking variation from the normal adult. The distribution of lipid was far more extensive than that seen in any other kidney. In addition to the large droplets always found in the convoluted portions of the tubule, the medullary loops

were also filled with medium-sized droplets throughout their length (fig. 3). The latter were somewhat smaller than those seen in the convoluted portions, but much larger than the fine granules occasionally found in the loop of Henle of the normal adult. The pregnant cat was the only one in whose kidney relatively large amounts of lipoid were observed throughout the loop of Henle. It is interesting to note that in these cats, whose kidneys contained more stainable lipoid than any of the others examined, the foetuses removed from their uteri showed the least amount of intracellular lipoid. This would indicate that the presence of stainable lipoid was not due to a circulating toxin, or any pathological factor, since if that were the case, the foetuses as well as the mother would be affected.

The cat starved for 14 days, which lost 44 per cent of its weight, had essentially the same lipoid picture in its kidneys as did the normal adult cat. The same was true of the kidneys removed from the cat fed on milk for 8 days and those from the cat after a heavy cream meal.

Although there is some controversy concerning the nature and function of the stainable lipoids in the liver, sections of the cat's livers were examined for lipoid and the relative amounts of stainable lipoids in the livers and kidneys of the same cats were compared. There was no correlation. While the amount of lipoid observed in the kidneys, with the exception of the very young and the pregnant cats, was essentially constant, the amount of lipoid seen in the liver varied almost directly with the weight of the adult animal. The livers of the kittens at term and 10 days old had extensive amounts of lipoid, whereas their kidneys showed the least amount of lipoid. The liver of the cat starved for 14 days was practically devoid of demonstrable lipoid, whereas its kidney appeared to be essentially identical with that of the heaviest cats examined. The kidney of the cat after the heavy cream meal showed no significant variation from the normal, although its liver was full of extremely large fat droplets which distended many of the hepatic cells and forced the cytoplasm to assume a crescentic shape.

The urine examined in the recent state, under the microscope, showed spherical refractile bodies, floating free, in every instance, with the exception of the urine from the kittens removed from utero. There was a considerable variation in the amount of droplets seen in the urine, but no method for determining it quantitatively or even relatively, except to judge the relative difference, was devised. However, it was clear that the urine of the pregnant cats contained more of these droplets than any other urine specimen. None were seen in the urine of the kittens at term and only a very few in the urine of the kittens 10 days old. No urine was taken from the foetuses 7.5 cm. long. No correlation could be determined between weight or sex of the cats and the number of droplets observed in the urine.

The objection that the presence of these droplets might be due to vaginal secretions or seminal fluid, especially in those cats from which the urine was milked by massaging the abdomen and collecting from the urethra, or from fecal contamination, is not valid, since in all but twelve of the thirty-one specimens examined, the urine was aspirated from the bladder in the open abdomen. The droplets were perfectly spherical and appeared to be lipoidal because of their refractile nature. They concentrated the dye when a dilute solution of alcoholic-alkaline scarlet red was added (fig. 4). The droplets appeared deep red, although the urine was only slightly tinged with the dye. The droplets did not disappear when acid was added to the urine and appeared to become somewhat larger and less numerous when the urine was boiled for a few minutes. When the urine was centrifugalized at very high speed (30,000 R.P.M in a Sharpless clarifier), the droplets were concentrated into the residual layer where they appeared to be larger than the droplets in an uncentrifugalized portion of the same specimen of urine. Under crossed Nicol prisms the droplets were not visualized. These droplets are interpreted to be lipid in nature. Three hundred cc. of collected urine was extracted for lipid. A small amount, about $\frac{1}{2}$ cc., not enough to perform any qualitative tests, was extracted.

DISCUSSION

The presence of large amounts of lipid in the renal tubule of the cat can hardly be thought of as being without special significance. It may exist there either as a fat deposit or in some functional capacity. It has been shown by Hartley that the fat kept available in the metabolic centers of the body is highly unsaturated and in a chemically active and mobile state. Mottram has shown that this is not true in the case of the lipoids in the renal tubule of the cat in which the fatty acids were shown to be highly saturated. If lipid were kept in the kidney available for metabolic consumption, one would expect that the amount would vary with the weight or obesity of the animal as it apparently does in the cat liver. However, there is no correlation between the weight of the cat and the amount of demonstrable lipid in its kidney. In the adult it appears to be constant in amount, except in the pregnant animal. Even in the starved animal, which lost 44 per cent of its body weight, practically exhausting the gross fat depots of the body, and whose liver was almost completely devoid of lipid droplets, the kidney had as much demonstrable lipid as the normal adult cat. In the cat after the heavy cream meal, on the other hand, the cells of the liver were distended by large lipid droplets, although the kidney showed no appreciable increase in amount of lipid over the kidneys of the other adult animals.

It would seem, however, that the variation in the amount of lipid is correlated, to some extent at least, with the functional activity of the kidney. In the kidney which was most active, that of the pregnant cat, the greatest amount of lipid was observed and the urine in this case showed the largest number of droplets. In the least active kidney, that of the foetus, the least amount of lipid was demonstrated and no droplets were found in the urine. It is generally accepted that the kidney of the pregnant animal carries a greater load than that of the normal animal. However, the assumption is not made here that per unit of kidney the foetal kidney is less active than the adult, but the foetal kidney has only to

excrete the end products of a simpler metabolic process and is exposed to less foreign matter than the adult. Hence, the foetal kidney probably has not developed all the excretory mechanisms found in the adult.

It has been shown that the lipoids in the renal tubule of the cat are highly saturated and not readily available for metabolic activity, yet such lipoids can be and are utilized by the animal organism. Because of the high caloric value of lipid, the excretion of it in the urine of the cat represents a loss of available energy and a fault in animal economy if one of the functions of the renal tubule of the cat is that of a lipid storehouse. The fact that the lipid is excreted into the urine speaks against the kidney being a fat depot and suggests that the renal tubule and the lipid contained therein play an active role in renal function in this animal. This idea is supported by the finding that there is a correlation between the amount of lipid demonstrable in the renal tubule and the state of renal activity of the animal. Whether the lipid acts as a vehicle for the excretion of certain substances,³ in solution or in chemical combination, or whether the lipid is excreted as such, as the end product of some metabolic process, because it cannot be utilized, is yet to be determined.

Whatever the exact function of the lipid in the renal tubule, it appears to be improbable that the renal tubule is a fat storehouse and the anatomical variations are very indicative of the fact that in the cat the lipid may play a role in renal function. If this is true then it is an instance in which excretion takes place through the tubule and is not filtered through the glomerulus. There is no question here concerning the excretion of lipid through the glomerulus and reab-

³Dr. Janet Travell, of the Department of Pharmacology, Cornell University Medical College, has made the very interesting observation (to be published) that after the injection of codeine, the cat excretes strikingly higher percentages of the alkaloid in the urine than does the dog or the rabbit. The base of this alkaloid is fat soluble. Although the large amount of lipid in the cat kidney and urine and the high percentage of excretion of a fat soluble substance in the urine may be an accidental and unassociated coincidence, it is highly suggestive. Work is contemplated to determine whether the renal lipid deposit plays any role in the excretion of codeine in the cat.

sorption by the tubule because lipid is never demonstrable in the glomerulus and is constantly found in the urine and convoluted tubules. The problem of glomerular filtration and tubular excretion is a controversial one which will not be discussed here. Whether the role of the lipid in the renal tubule of the cat indicates that the kidney functions in a manner different from that in other animals or whether the difference is only one of degree remains to be determined. Inasmuch as very fine lipid granules are demonstrable in the renal tubule of other mammals the latter possibility may be true. It may also be true that the lipid picture found in the kidney and urine of the cat would also be found in other purely carnivorous animals, especially the felines, if their kidneys and urine were examined.

SUMMARY AND CONCLUSIONS

1. Large amounts of lipid droplets are demonstrable in the convoluted portions of the renal tubule of normal adult cats, and are absent in the other portions of the tubule.

2. In very young kittens and fetuses the lipid is found only at the junction of the proximal convoluted tubule and the descending limb of Henle's loop, and seems to extend as very fine granules toward the glomerulus as the cat grows older.

3. The pregnant cat shows the most extensive lipoidal deposit.

4. Lipoid droplets are found in the lumina of the convoluted tubules and also in the urine of adult cats. It is assumed that the cats excrete the lipid droplets from the cells of the tubule into the urine. In very young kittens, showing little lipid in the tubule, little or no lipid is found in the urine.

5. There is no correlation between the amount of lipid demonstrable in the liver and that in kidney of the cat.

6. There is no correlation between the weight of the animal and the amount of lipid in the renal tubule, but there apparently is a correlation between renal activity and the renal lipid deposit.

7. It is suggested that the renal tubule of the cat is not a fat storehouse, but that the lipoid in the tubule has an as yet undetermined role in renal function.

8. It is also suggested that the lipoid picture in the cat's kidney and the role played by the lipoid in renal function may not be characteristic of only that animal but that it may exist in other truly carnivorous animals to some degree, and that it may exist in a much lesser degree in other mammals.

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PLATE I

EXPLANATION OF FIGURES

1 Photomicrograph of the cortex of the adult cat kidney stained for lipoid, showing the convoluted tubules filled with droplets which photograph black. Note that the loops of Henle, the collecting tubules, and the glomeruli are free of lipoid droplets. The same picture is seen in all the adult kidneys, including that of the starved animal and the one after a heavy cream meal.

2 Photomicrograph of the kidney of a kitten 10 days old. Shows medium-sized lipoid globules at the junction of the proximal convoluted tubule and the descending limb of Henle's loop.

3 Photomicrograph showing fat droplets in the loop of Henle taken from the kidney of a pregnant cat.

4 Photomicrograph of concentrated cat's urine in which the lipoid droplets have concentrated the dye added to it.

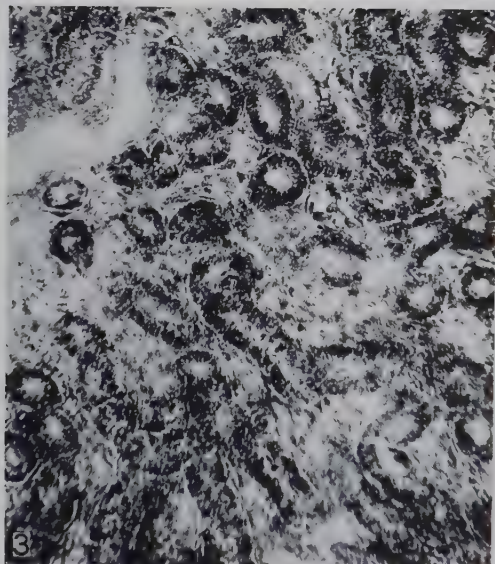
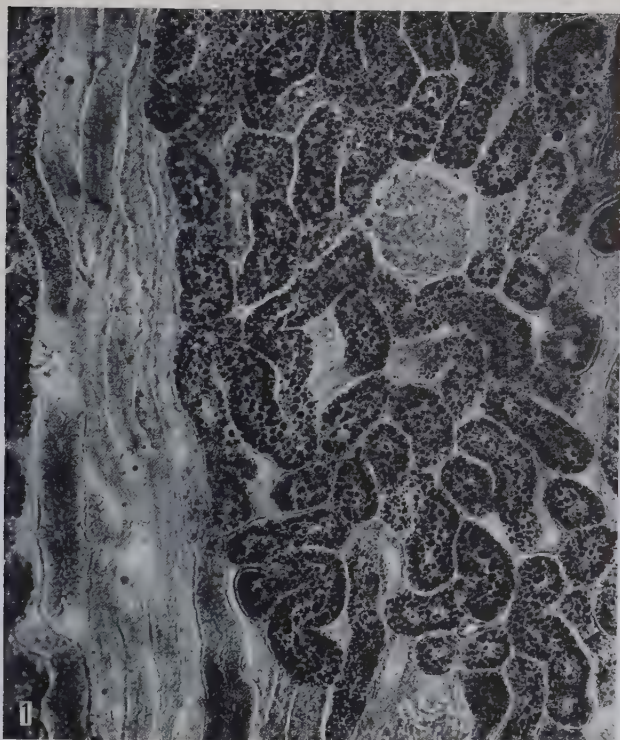


PLATE 2

EXPLANATION OF FIGURES

5 Photomicrograph showing the cortex of the kidney of a cat whose tubules have been distended by ligation of the ureter. Note the lipoid droplets in the lumina of the tubules.

6 Drawing of part of the same field as in figure 5.



THE GOLGI APPARATUS IN THE DEVELOPING TOOTH, WITH SPECIAL REFERENCE TO POLARITY

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SIX FIGURES

The constant and regularly polarized condition of the Golgi apparatus in many types of epithelial and glandular cells led Golgi and Cajal to suggest the possibility of using structure as an indicator of secretory polarity. Cowdry was one of the first to apply this suggestion when he made a study of the mechanism of secretion in the thyroid gland. By using the Golgi apparatus as an indicator of secretory polarity he found that some of the cells secreted directly into the blood stream while others emptied their secretion into the lumen of the follicles. Unhappily, this and similar studies, in which the Golgi apparatus has been used to indicate a reversal in direction of secretion, have not been generally confirmed and some writers have even expressed doubt as to the advisability of ever using the Golgi apparatus as an indicator of physiological polarity.

In view of the increased efforts to use the Golgi apparatus as a morphological indicator of different physiological states of the cell, it seems that the developing tooth, particularly the enamel organ, might offer excellent material to test the hypothesis that the Golgi apparatus is an indicator of secretory polarity. It is well known that the ameloblasts reverse their polarity just preceding the formation of enamel. Accordingly, it would be of considerable importance to determine whether or not the Golgi apparatus reverses its position concomitantly with the other cytological structures of the cell.

MATERIAL AND METHODS

The material for this study consists of the developing molar teeth of rats, 1 to 5 days old. For general histological and cytological studies the lower jaw was fixed in Zenker's Helley's, Bouin's, and Flemming's fluids, then stained in Delafield's and Heidenhain's hematoxylin. It was unnecessary to decalcify the tissue following fixation for 1 week in Bouin's fluid. For a study of the Golgi apparatus Nasonov's method proved successful. In some cases the mitochondria were also impregnated following Nasonov's method and were consistently revealed after the method of Regaud. It was unnecessary in the Nasonov and Regaud techniques to employ a special decalcifying fluid.

DESCRIPTION

Since the general embryology of the teeth is so well known it will be unnecessary for us to review it at length here. It will suffice to point out that the teeth have a dual embryological origin, the enamel organ being formed from the ectoderm of the oral epithelium and the dental papilla from the underlying mesoderm.

The enamel organ originates as an invagination of the epithelium which gives rise to a sac-like structure filled with cells which are continuous with the oral epithelium. It finally becomes isolated and much flattened by the pushing up of the dental papilla over which it forms a cap. The cells in contact with the dental papilla, known as inner enamel cells, become much modified in shape and size and are the enamel forming portion of the organ. The enamel cells not in contact with the dental papilla are known as outer enamel cells and are less modified than the inner ones. The tissue in the interior of the enamel organ (enamel pulp cells), although ectodermal in origin, resembles to some extent mesenchymal connective tissue and in the rat contains capillaries.

The formation of enamel and dentine may be demonstrated in the developing molar teeth of 1- to 5-day-old rats. The enamel is deposited first at the apex of the crown and then

downward toward the root of the tooth so that in an unerupted tooth a marked but gradual transition between the short inactive ameloblasts and the taller active ameloblasts can be observed. Figure 1 represents a section through such a region, showing the striking change in size between the active and inactive enamel forming cells. It will be recalled that during the transition from the inactive to the active ameloblasts the nuclei migrate from their position at the base of the inactive ameloblast to the opposite end of the cell, i.e., the end directed toward the enamel pulp. In this way the original basal or mesenchymal end of the inactive ameloblast has become modified as the free or discharging end of the functional ameloblast as indicated by the laying down of enamel. The Golgi apparatus has a net-like form in the inactive ameloblast and is located between the nucleus and the enamel pulp end of the cell, or, in other words, on the opposite side of the nucleus from the odontoblasts and the dental pulp (fig. 1). This position of the Golgi apparatus is similar to that of the basal cells of the Malpighian layer of the oral epithelium from which they have descended (fig. 3). In those ameloblasts of the zone of changing polarity a striking migration of the Golgi apparatus to the opposite pole of the nucleus is observed. It will be noted that the Golgi apparatus migrates in the form of lengthened cords or strands along the sides of the nuclei to take up a position in the active ameloblasts adjacent to the nucleus of the discharging end of the cell which represents the basal end of the inactive ameloblasts. This change in polarity of the Golgi apparatus is rather abrupt (fig. 1). The Golgi apparatus in the ameloblasts immediately following the reversal in polarity again assumes a rather compact net-like structure which gradually becomes less compact as the cells rapidly grow in height. In the very tall ameloblasts which are usually in association with a well-developed layer of the enamel, the network of the Golgi apparatus becomes drawn out into long anastomosing strands which extend in some instances throughout the greater part of the cytoplasm between the nucleus and the discharging end

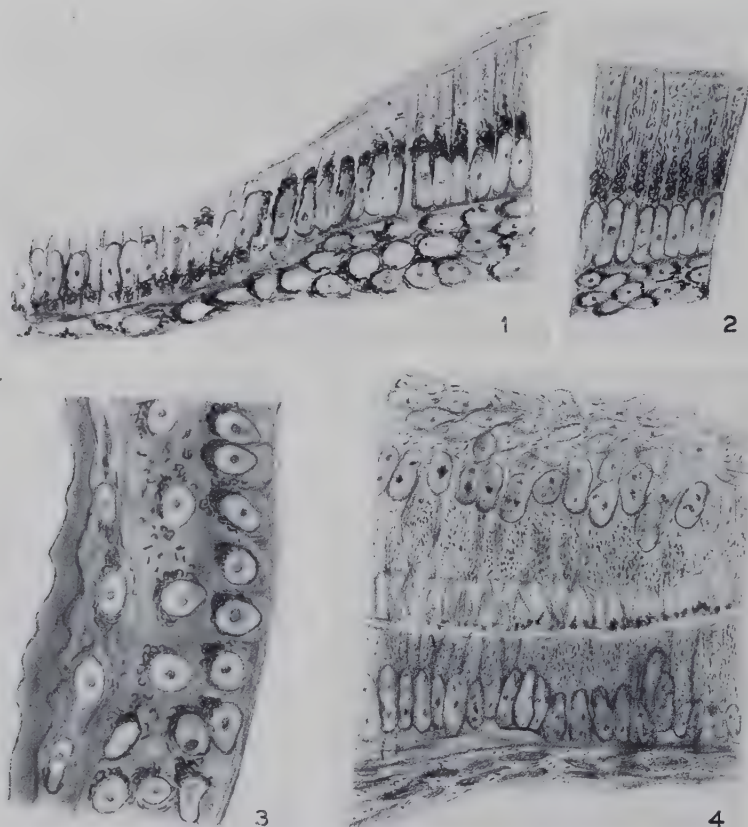


Fig. 1 Ameloblasts in zone of transition from inactive to active condition. The position of Golgi apparatus in active cells is reversed from that of inactive cells. Enamel pulp with Golgi apparatus. Molar tooth of rat 1 day old. Nassonov technique.

Fig. 2 Active, elongated ameloblasts with Golgi apparatus distended. Molar tooth of rat 5 days old. Nassonov technique.

Fig. 3 Oral epithelium, Golgi apparatus polarized in basal layer as in inactive ameloblasts. Stratum germinativum to right. Molar tooth of rat 2 days old. Nassonov technique.

Fig. 4 Mitochondria in cells of developing tooth. Odontoblasts and dentine pulp cells above, ameloblasts and enamel pulp cells to left. Molar tooth of rat 3 days old. Regaud technique.

of the cell (fig. 2). This striking change in the form of the Golgi apparatus might be interpreted to represent an increased activity on the part of the ameloblasts (secretion of enamel?) rather than the result of a mechanical displacement caused by the extreme lengthening of the cells. In any case, the Golgi apparatus remains constantly polarized on the side of the nucleus next to the enamel (fig. 5) in a general position comparable to the so-called secretogenous zone of many gland cells.

In the cytoplasm of the actively secreting ameloblasts there are often found small granules which are probably connected with the formation of enamel. We could not, however, observe that they were formed directly in association with the Golgi apparatus. Between the nucleus and the end of the cell adjacent to the enamel pulp is often found a small globular body which appears brown or black following treatment with osmic acid. In ordinary histological preparations this body is dissolved, leaving a clear vacuole (fig. 6).

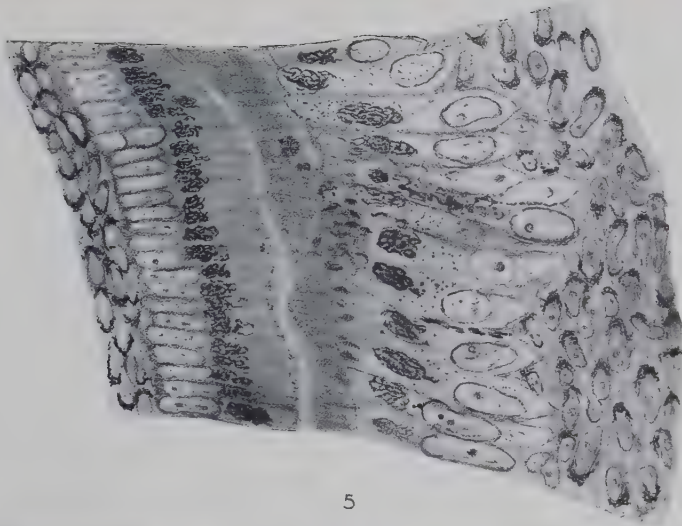
The pulp cells of the enamel organ which have been derived from the stratum Malpighii of the oral epithelium undergo a very striking change from their former stratified condition. The tissue in the interior of the enamel organ becomes separated and changed into irregular stellate anastomosing cells which resemble in some ways those present in mesenchymal tissue (figs. 5 and 6). Each of the cells possesses a compact localized Golgi apparatus (fig. 5) typical of the Golgi apparatus of connective tissue cells. The intermediate layer of enamel pulp cells, i.e., those subjacent to the ameloblasts, has retained its more or less flattened stratified epithelial-like character. The Golgi apparatus of these cells is localized in the form of a compact network usually at one side of the nucleus. However, as in the dental pulp cells, not all are polarized in the same direction. Whether the lack of uniform polarity of the enamel pulp cells in any one direction is due to a change in their position during dedifferentiation from the oral epithelium or to a migration of the Golgi apparatus within them has not been determined.

The odontoblasts which represent the layer of rearranged cells¹ bordering the enamel layer, like the ameloblasts vary considerably in height (figs. 4, 5, and 6). In general they appear wider and not as tall as the ameloblasts. They possess a well-formed net-like Golgi apparatus which is very easily demonstrated by osmic acid methods (fig. 5). In fact careful study of ordinary histological preparations often reveals the negative image (canalicular apparatus) of the Golgi apparatus in these cells (fig. 6). It is localized in a position between the nucleus and the discharging end of the cell, a condition so characteristic of gland cells.

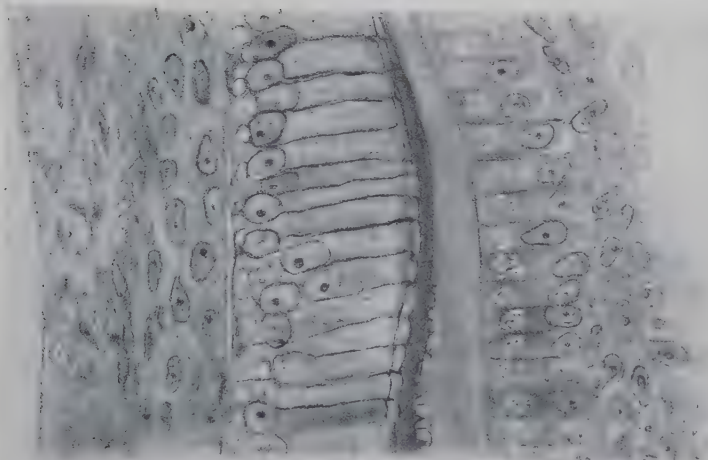
Here, as in the ameloblasts, well-defined secretory granules may be observed which may be associated with the formation of dentine. We have not seen that they are formed in the filaments of the Golgi apparatus. In the underlying dental pulp cells the Golgi apparatus resembles, in a general way, that which has been described for the enamel pulp cells. It will be noted that those cells immediately adjacent to the odontoblasts are arranged into a more or less compact, flattened layer (intermediate layer). These, together with the deeper cells, which represent typical mesenchymal tissue, likewise contain a compact net-like Golgi apparatus typical of connective tissue cells. These cells, while probably individually polarized, are not so in any one given direction. However, it is interesting to note that those which have formed the odontoblasts have all become polarized in a common direction (fig. 5).

Mitochondria in the ameloblasts, odontoblasts, and pulp cells do not show any striking characteristics. They are in the form of filaments, short rods, and granules which are fairly numerous and distributed throughout the greater part of the cytoplasm (fig. 4). However, the mitochondria in both

¹It may be recalled that the odontoblasts have originated from mesenchymal connective tissue cells which line up in a single layer, elongate, and become very similar to typical columnar epithelial cells. Such a transformation has been recently described by Papanicolaou ('33) for the guinea pig uterus where the sloughed off columnar epithelium is replaced from the underlying connective tissue cells. (*Am. Jour. Obst. & Gynec.*, vol. 25.)



5



6

Fig. 5 Golgi apparatus in cells of developing tooth. Odontoblasts and dentine pulp cells to right, ameloblasts and enamel pulp cells to left. Molar tooth of rat 3 days old. Nassonov technique.

Fig. 6 Developing tooth, orientation as in figure 5. Odontoblasts show negative image of Golgi apparatus. Ameloblasts with small vacuole between nucleus and underlying pulp cells. Molar tooth of rat 5 days old. Zenker fixation, Delafield's hematoxylin, eosin.

the ameloblasts and odontoblasts seem to show little change in form in the region of the Golgi apparatus.

Diplosomes have been found in the active ameloblasts on the same side of the nucleus as the Golgi apparatus. Unfortunately, they have not been observed by us in the outer ameloblasts or in the oral epithelial cells but they have been described by Zimmerman (1898) in stratified epithelium as being located distal to the nucleus, i.e., where the Golgi apparatus has been observed by us in the oral epithelium.

DISCUSSION

The determination of polarity in cells constitutes one of the many perplexing problems of biology. We can study only its visible expression which is, in many cases, both structural and functional, apparently on the one hand as a localized grouping of cell components, on the other as a difference of functional or metabolic activity with respect to the organic axis of the cell.

The position of cytoplasmic components such as the nucleus, centrosomes, diplosomes, secretion granules, yolk bodies, etc., as well as the position of the cells in relation to their nutrition, has been taken to indicate the structural expression of the physiological polarity of the cell. However, Lillie ('09) points out "that polarity is not a result of the position of the nucleus or of any configuration of granules" but is due to some heterogeneous physical chemical properties of the ground substance.

However, more recently the position of the Golgi apparatus has been used as a cytological indicator of the physiological state and polarity of the cell. Golgi ('09), D'Agata ('10), Basile ('14), Cajal ('15), Cowdry ('22), Reiss ('22), Courrier and Reiss ('22), Jasswoin ('24), Timofejev ('25), Ludford and Cramer ('28), and Giroud ('28), have all described a reversal in polarity of certain types of cells as indicated by the position of the Golgi apparatus. In many of the above-mentioned cases the physiological condition of the cells in which the reversed position of the Golgi apparatus took place

was not known. Accordingly, these results are open to question since it has been suggested that the reversed position of the Golgi apparatus in the cells may be due to a mechanical displacement. But the enamel organ offers, it seems to us, a more conclusive test than most of these cases in which a reversal in polarity has been described. It differs in that we are dealing with a case where the known original basal end of the cell becomes the discharging end and remains such throughout the development of the tooth. It is difficult to believe that the reversal in polarity of the Golgi apparatus in this particular case is not associated directly with a change in the polarity of the cell. It is true no doubt that as the cells beginning to secrete grow rapidly in height, the Golgi apparatus is stretched and extends proportionally, but this does not account for its migration to the other side of the nucleus.

Cajal ('14) first described the Golgi apparatus of the odontoblasts and dental pulp cells as a compact localized net. At about the same time Massenti ('14) described a diffuse network in ameloblasts, enamel pulp cells, odontoblasts, and dental pulp cells. The observations of Jasswoin ('24) and of Timofejev ('25) disagree with the findings of Massenti with whose work they apparently were unfamiliar.

Jasswoin and Timofejev describe a polarized condition for the ameloblasts and odontoblasts and further note the change in polarity reemphasized here. Schour ('32), in his chapter 'The Teeth' in Cowdry's *Special Cytology*, suggests the necessity for a reexamination of the polarity of the ameloblasts. It may be definitely stated that there is an obvious morphological reversal of polarity in the ameloblasts corresponding to the change in functional polarity. These facts can be interpreted to favor the secretion theory of enamel formation.

CONCLUSIONS

1. The Golgi apparatus and mitochondria of the oral epithelial cells, ameloblasts, enamel pulp cells, odontoblasts, and dental pulp cells have been described.

2. The position of the Golgi apparatus has been used as a criterion of morphological polarity.

3. A change in morphological polarity following a change in functional polarity has been described for the ameloblasts.

4. This change in morphological polarity involves the Golgi apparatus (and probably the diplosomes) which migrates around the nucleus to the opposite pole of the cell.

5. Thus the Golgi apparatus of active ameloblasts resembles that of glandular cells in that it is located at the active end of the cell.

6. This may indicate that enamel formation is by secretion rather than by transformation of the ameloblasts.

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A METHOD FOR STUDYING ADRENAL AND OTHER LIPOIDS BY A MODIFIED GELATIN EMBEDDING AND MOUNTING TECHNIQUE

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The lipoids of the adrenal are difficult to study histologically because of their solubility in the reagents used in paraffin and celloidin embedding techniques. The resulting vacuoles indicate the former resting places of the fat-like substances, but space does not yield much satisfactory information to the inquirer. Protective substances, such as osmic acid, can be used, but they affect only a limited group of chemical radicals, the most common of which is oleic acid.

Frozen sections of fresh or formalin-fixed tissues eliminate the solubility factor, but have other disadvantages. Serial sections are hard to get unless the sections are too thick to be of cytological value. Thin sections fragment easily during the handling necessary in staining and mounting, and ice crystals tear the tissues internally.

Gelatin embedding has been used by Nicolas (1895), Olt ('06), and Gaskell ('12) to improve the freezing technique, but these authors had difficulties in the subsequent handling of the tissues.

Our technique consists of:

1. A double embedding in 5 per cent and then 10 per cent gelatin; refrigeration of the embedded material and cutting into blocks.

The fixed material is washed for 4 hours or more to remove all formalin or other fixative and is then put in 5 per cent gelatin in an incubator at 35° to 37°C. for 24 hours. It is transferred to 10 per cent gelatin for 12 to 16 hours at the same temperature. The tissue is then embedded in 10 per cent gelatin (a Petri dish is convenient) and put in a refrig-

erator for 3 hours. Thicker gelatin than 10 per cent is not advisable.

2. Fixing the gelatin block in formalin.

The blocks of tissue in gelatin are cut out and dropped into 10 per cent formalin (4 per cent formaldehyde) for several hours to make the gelatin insoluble in water. They can be preserved in this fluid indefinitely. The gelatin blocks of many organs can be put to good use in studying the tissue relationships within organs, both by observation under a dissecting binocular and by supplementary fine dissections.

3. Freezing the gelatin block and sectioning.

Before sectioning, the blocks are rinsed in water and trimmed close to the tissue. Freeze with dry CO₂ gas until the entire block is uniformly white and then allow it to thaw until the knife no longer shaves the ice, but gives a real slice. Set the indicator for the desired thickness and cut rapidly while conditions are favorable. We have obtained 5 μ sections with some tissues.

4. Preserving the sections (serially, if necessary).

When serial sections are needed, use a piece of filter paper marked off into numbered squares, placed in a flat dish, and moistened with distilled water. Take each section off the knife with a camel's hair brush and place on the proper square. If representative sections only are wanted, they can be put in distilled water in a Syracuse watch glass. The cut sections may be kept indefinitely, provided the water is replaced by 10 per cent formalin or a little concentrated formalin added to the water as a preservative.

5. Mounting the sections on slides with 1 per cent gelatin and fixing the mounted section in 10 per cent formalin solution.

To mount, float the section on to a clean slide under distilled water and remove the slide from the water, holding the section in place with a fine brush or a pointed glass rod with the tip bent at right angle (Gaskell, '12). Surface tension helps in flattening the section. The excess of water is carefully drained and wiped off and a drop or two of 1 per cent gelatin solution run under the section (modified Masson, '28). Any excess is drained off. About 5 minutes of dry heat at 33° to 37°C. will dry the slide, which is then put in a

10 per cent formalin solution for at least 10 minutes to fix the gelatin. If properly done, the section will not come off, even when the slide is held under running tap water. At this stage the slide may be kept indefinitely in 10 per cent formalin.

6. Staining.

The usual fat stains can be used, alone or in combination with a wide variety of the water soluble stains. We have used sudan III, sudan IV, Nile blue sulphate, and osmic acid for the lipoids. Many unusual combinations of stains have been used by this method. For example, an excellent preparation was obtained with a section of human adrenal stained with modified Laidlaw's silver for nerve axons, Scharlach R for lipoids, and Mallory 'C' for connective tissue and nerve cells. The birefringent lipoid of the adrenal, which frequently does not take up stain, could be seen in polarized light in spite of the foregoing procedures.

7. Putting on cover-slips with 'glychrogel.'¹

One of the objections to the use of frozen sections is the lack of a satisfactory mounting medium. Even after ringing with dammar or asphalt, a section mounted in glycerin or glycerin jelly can be spoiled by cleaning after the use of immersion oil.

After some research, we developed a mounting medium which we call 'glychrogel.' It flows freely at a temperature of 30°C. and can be used for long periods at room temperature. It will hold a cover-slip in place after 1 hour, and after 24 hours it is impossible to remove the cover-slip by force. It is advisable to use a slight excess of glychrogel in mounting the covers, so that a protective film may form. Any great excess can be easily removed 24 hours after mounting by washing with water and wiping with a towel. The solution has a satisfactory refractive index, since we have used oil immersion and polarized light with no apparent distortion.

¹ '*Glychrogel*' mounting solution. To make 100 cc., take 20 cc. glycerin, 3 gm. Knox granulated gelatin, 0.2 gm. chrome alum, and 80 cc. distilled water. Dissolve separately the chrome alum in 30 cc. of the water by aid of heat and the gelatin in the remaining 50 cc. of water. Combine the 20 cc. of glycerin and the gelatin solution while the latter is still warm. While stirring above mixture, add the 30 cc. of warm chrome alum solution. When thoroughly mixed, filter and add a crystal of camphor as a preservative. If bubbles are present, warm the solution. If the solution congeals in a cold room, put it in an oven at 37° until it flows freely again. Keep the bottle well stoppered to prevent evaporation.

It may be necessary to ring the slides with dammar or balsam if they are to be kept permanently, as we are not sure of the amount of evaporation over a long period of time. However, 36 hours of dry heat and 9 weeks at room temperature have not affected the slides in any noticeable way. For the immediate study of sections glychrogel is excellent, especially if oil immersion is to be used. Ordinary frozen sections can be mounted in glychrogel if speed is necessary.

We are using the foregoing method and modifications of it for the study of the microchemical behavior of the various lipoids of the adrenal in relation to their changes in disease and their physiological significance. Sections of brain, ovaries, testes, nephrotic kidneys, and other 'visible-lipoid' containing tissues have also been studied satisfactorily.

I wish to extend my thanks to Miss Marie Norkus for her assistance in the development of this method.

SUMMARY

A method for embedding, cutting, mounting, staining, and covering visible-lipoid containing tissues is described. The technique is briefly: the embedding of the tissue in gelatin; fixation of the gelatin block in formalin; sectioning after freezing; fixing the sections firmly on slides with dilute gelatin followed by formalin solution; staining by usual and rare combinations of stains, and, finally, mounting in a new medium, 'glychrogel.' The preparation and properties of this mounting solution are given.

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DIFFERENTIAL EFFECT OF PROLAN IN DECREASING THE POTENCY OF THE HYPOPHYSIS IN NORMAL AND CASTRATE RATS ¹

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The effect of oestrin in diminishing the production and storage of the sex stimulating complex of the pituitary as determined by direct assay has been clearly established by Meyer, Leonard, Hisaw, and Martin ('30, '31, '32). This oestrin-pituitary relationship has been confirmed and extended by many others, particularly by Moore and Price ('32). Since oestrin decreased the amount of the gonadotropic hormone in the anterior pituitary, it seemed probable that the injection of an extract which increased the sex hormone output of the gonads would also diminish the potency of the pituitary. Furthermore, if the animals were castrated, then the gonadotropic principle would be without effect upon the hypophysis.

A few preliminary experiments had been made using prolan (pregnancy urine extract) as a gonadotropic stimulator, when a paper by Kuschinsky ('31) appeared in which he showed that prolan decreased the potency of the hypophysis of normal female rats, but was apparently without effect in the castrate females. The problem was discontinued for some time, but with the newer discoveries of the action of prolan in combination with hypophyseal hormones (Evans, Meyer, and Simpson, '32; Leonard, '32), the problem was taken up with the idea of confirming the findings of Kuschinsky. In his work only female rats were used, but in this work it was extended to include both sexes.

¹ Aided by a grant from the National Research Council, Committee on Problems of Sex, administered by Dr. P. E. Smith.

² National Research Fellow in Biological Sciences.

Adult male and female rats were injected with an extract of pregnancy urine prepared by the alcohol precipitation method of Zondek or with Follutein, a pregnancy urine preparation of Squibb & Co.³ For controls, animals of nearly the same age and weight (not necessarily litter mates) were used. It was noted that usually the prolant injected animals did not grow as well as the uninjected controls, so before the experiment was begun, the heavier rats were selected to receive the treatment. In this way, an endeavor was made to keep the body weights of the experimental and control donors within a range of 20 gm. of each other at the time of implantation of the hypophysis. The exact dosages and length of treatment are given in the tables. At the termination of the injections, the animals were killed by decapitation, their pituitaries removed and each implanted into an immature female mouse 20 to 22 days old. To secure as uniform data as possible, litter mate mice were used and within each litter only the experimental and control donors of the same sex were compared. After a lapse of 4 days from the time of implantation, the mice were killed and the ovaries weighed (as a criterion of the degree of stimulation by the hypophysis of the treated and control animals). The tables 1 and 2 indicate that in both the males and females a marked decrease in the gonad-stimulating potency of the pituitary was produced by prolant.

The general effect of prolant on adult males and females has not been investigated in detail by other workers and will be considered at another time.

In the males, a slight decrease in size of the testes and a very great increase of the seminal vesicles and prostate gland were noted. In the females, sometimes very large ovaries were produced weighing up to 200 mg.; in other cases they were not much larger than those of the controls. This point is not in exact agreement with Collip et al. ('31), who state that pituitary-like placental extracts (which are potentially similar to prolant) cannot produce ovaries of such large size.

³ Dr. J. A. Morrell, of E. R. Squibb & Sons, kindly furnished the Follutein.

These large ovaries contained follicular cysts, lutein cysts, and many large corpora lutea. The uteri of the injected females were enormously increased, sometimes weighing as much as 0.6 gm. Both the large size of the uterus in the females and of the accessory organs of the male give conclusive evidence of the great overproduction of the specific sex hormones.

TABLE 1

Decreased potency of the anterior pituitary of normal female rats following injections of extracts of pregnancy urine

TREATMENT OF DONOR RATS			RECIPIENTS—AVERAGE OVARIAN WEIGHTS			
Extract	Amount	Period	Litter	Control	Treated	Per cent decrease
	<i>R.U.</i>	<i>Days</i>		<i>Mg.</i>	<i>Mg.</i>	
Follutein	281	34	L	4.3	3.0	30.2
Follutein	281	34	M	4.8	2.4	50.0
Follutein	100	10	O	8.9	6.95	21.8
Follutein	100	10	P	9.3	6.1	34.4
Follutein	100	10	Q	7.4	5.0	32.4

Total number of animals = 15

Average per cent decrease = 33.7

TABLE 2

Decreased potency of the anterior pituitary of normal male rats following injections of extracts of pregnancy urine

TREATMENT OF DONOR RATS			RECIPIENTS—AVERAGE OVARIAN WEIGHTS			
Extract	Amount	Period	Litter	Control	Treated	Per cent decrease
		<i>Days</i>		<i>Mg.</i>	<i>Mg.</i>	
Prolan	9 cc. = 45 cc.	9	A	11.3	6.6	41.6
Follutein	320 R.U.	13	C	13.0	8.4	35.4
Follutein	320 R.U.	13	D	8.9	5.0	43.8
Follutein	320 R.U.	17	E	5.7	4.5	20.0
Prolan	4.3 cc. = 42.3 cc.	17	F	13.0	4.6	64.6
Prolan	4.3 cc. = 42.3 cc.	17	G	7.5	3.8	49.3

Total number of animals = 20

Average per cent decrease = 42.4

Whereas the injections of prolan cause a reduction of 20 to 60 per cent in the potency of the normal male and female pituitaries, this decrease did not occur if the treated animals were castrated. Normal rats of both sexes were castrated for varying periods of time as indicated in tables 3 and 4. They were selected for injections on the basis of body weights,

so that there was no greater variation than 20 gm. between experimental and control donors. The animals were injected with amounts of prolan and for a sufficient length of time that would give a marked decrease in potency in normal animals. The method of implantation into litter mate mice was similar to that in the preceding group comparing only castrated treated animals with castrated untreated animals of

TABLE 3

Ineffectiveness of prolan in decreasing potency of anterior pituitary in castrated female rats

TREATMENT OF DONOR RATS				RECIPIENTS—AVERAGE OVARIAN WEIGHTS			
Number days castrated	Extract	Amount	Period	Litter	Control	Treated	Per cent decrease
			<i>Days</i>		<i>Mg.</i>	<i>Mg.</i>	
233	Prolan	4.6 cc. = 47.6 cc.	14	L1	37.5	32.4	13.6
5	Prolan	11 cc. = 165 cc.	11	M1	9.6	8.5	11.4
30	Prolan	3 cc. = 60 cc.	12	O1	19.6	17.4	11.4
Total number of recipients = 11				Average per cent decrease = 12.1			

TABLE 4

Ineffectiveness of prolan in decreasing potency of anterior pituitary in castrated male rats

TREATMENT OF DONOR RATS				RECIPIENTS—AVERAGE OVARIAN WEIGHTS			
Number days castrated	Extract	Amount	Period	Litter	Control	Treated	Per cent decrease
			<i>Days</i>		<i>Mg.</i>	<i>Mg.</i>	
234	Prolan	4.6 cc. = 47.6 cc.	14	A1	35.3	32.0	9.37
79	Prolan	3 cc. = 60 cc.	12	B1	25.1	24.5	2.37
79	Prolan	3 cc. = 60 cc.	12	C1	25.6	25.4	0.0
Total number of animals = 13				Average per cent decrease = 3.7			

the same sex within each litter of recipients. The tables 3 and 4 give the specific treatment for each group.

In contrast to the effect of prolan on the normal male and female rat pituitaries, the hormone was without significant effect on the gonad stimulating power of the castrate animals' hypophyses. The injected prolan was free of oestrin, for the castrate females did not show any vaginal changes indicative of the female sex hormone. In two cases the

vagina was sectioned for mucifying quantities of oestrin (R. Meyer and W. Allen, '31), and this was also negative. Evidently, the effect of prolan on the hypophysis was due to a stimulation of the gonads in the normal animals to form an excess secretion of the sex hormones (oestrin in female, male hormone in the male), which in turn depleted the hypophysis.

The actual ovarian weights of the mice produced by the castrated animals' hypophyses are much heavier than those of the control normal animals—a fact which Engle ('29) and others pointed out some years ago. Also the length of time of castration has a definite effect on the potency of the pituitary, which would make the actual ovarian weights greatest in the animals castrated the longest.

DISCUSSION

The experiments just presented confirm the excellent work of Kuschinsky, in which he demonstrated that prolan causes a decrease in potency of the female rat pituitary, but fails to do so when the animal is castrated. This has been extended to the male rat, where the results were the same. From the earlier work of Meyer, Leonard, Hisaw, and Martin, the female sex hormone was found capable of decreasing the potency of the hypophysis of the castrate male and female rats and normal, immature female rat as indicated by direct assay of implantation. Since prolan is known to produce the sex hormone in excess when injected into normal rats and that it fails to modify the gonadotropic hormone content of the hypophysis of castrate rats, it is quite logical to conclude that prolan acts on the hypophysis only by way of the gonad. Prolan seems not to be a hypophysis stimulator per se.

It should be pointed out that this failure of prolan to affect the hypophysis of the castrate rat holds only for the sex stimulating complex as indicated by direct assay. If another test for hypophyseal hormone should be applied or changes in the gland itself observed, the results could possibly be different. Doctor Severinghaus in this laboratory is engaged in cytological studies of the hypophysis of rats treated in this manner.

Evidence that prolan has no direct effect on the hypophysis seems plausible from the work of White and Leonard ('33) on rabbits. Female rabbits hypophysectomized in heat and injected within an hour with minimal ovulating doses of prolan or pituitary extract revealed that it required one-sixth to one-third more of pituitary extract and one-half more of prolan to induce ovulation. In spite of this slight increase, it does not seem to indicate that the ovulating substances are altering the intact gland when inducing ovulation, but are acting directly on the gonad.

Finally, a consideration of the interaction of prolan with the circulating hypophyseal hormones may be of importance in this relationship. Recently, Evans, Meyer, and Simpson ('32) presented evidence that prolan could activate a growth hormone preparation and apparently transform it into a sex stimulating hormone. It has also been demonstrated (Leonard, '32) that a hypophyseal sex hormone fraction, when combined with prolan, can produce in an immature female rat a larger ovary than can be predicted by an addition of the individual hormonal effects. While it is true that prolan can alter the effects of hypophyseal extracts or secretions, it seems to be equally as true that prolan does not act directly on the intact hypophysis to alter its secretions, but only by way of the gonads.

SUMMARY

Confirmatory data are presented to show that prolan can deplete the gonad stimulating complex of the pituitary of normal female rats, but fails to do so if the animal is castrated. These findings have been extended to include the male. The effect of the continued injections of prolan on the normal males and females is to cause a great overproduction of the sex hormones which is reflected in the enormous size of the accessory organs of reproduction. It is concluded that prolan decreased the potency of the pituitary by the excess production of the sex hormones. It has been shown by the author and his co-workers that the sex hormones are re-

sponsible for the decreased gonad stimulating power of the hypophysis.

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THE DISTAL PROJECTION OF THE ENDOLYMPHATIC SAC IN HUMAN EMBRYOS¹

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TWENTY-FIVE FIGURES

The endolymphatic duct in the mammalian embryo is described conventionally as ending in a simple dilated extremity, the endolymphatic sac; and so far as the writer knows, it is not stated in any textbook that from the distal aspect of the endolymphatic sac in young human embryos there regularly extends a considerable projection the length of which may actually exceed that of the endolymphatic duct.

The projection has, however, been figured by several investigators as it appeared in models of the human membranous labyrinth. Thus in Streeter's ('07) figure of the labyrinth in the embryo of 30 mm., there is a knob-like protuberance on the dorsum of the endolymphatic sac; in Macklin's ('21) models of the 43-mm. (CH) embryo, the projection is long and thin; and in those by Bast ('30) of the 135-mm. and the 147-mm. embryos it is short, and of triangular outline. Doctor Macklin described what he observed, recording briefly that the dorsal extremity of the broad flattened saccus "is a very slender filamentous process containing an almost imperceptible lumen."

The present study is concerned with the appearance of this process in a graded series of human embryos.

¹ Contribution no. 190 from the anatomical laboratory, Northwestern University Medical School. Paper read at the New York City meetings of the American Association of Anatomists, February, 1932; abstract, *Anatomical Record*, vol. 52, no. 1, pp. 2-3, February 25, 1932.

MATERIAL AND METHODS

The observations recorded herein are based upon a study of the mammalian series available in the Harvard Embryological Collection, and constitute part of a more comprehensive comparative study on the development of the ear in vertebrates.

For the study of the endolymphatic sac and its projection, 141 embryological series were examined, distributed among eleven species of mammals as follows: forty-eight series of human embryos, from 10 to 74 mm. in length (CR)²; twenty-two of the cat, from 6 to 39 mm.; five of the dog, from 12.5 to 17 mm.; seventeen of the rabbit, from 5 to 33.8 mm.; six of the guinea-pig, from 6.1 to 30 mm.; nine of the rat, from 6 to 24.8 mm.; fourteen of the pig, from 7.8 to 48 mm.; three of the calf, 14.2 to 25.2 mm.; ten of the sheep, from 6.5 to 48.4 mm.; two of the deer, 9.8 and 18.6 mm.; seven of the opossum, from 13 to 26 mm.

Two series ('29 mm. and 40 mm.) were modeled by the Born wax-plate method, at a magnification of 250 diameters.

Twenty-five of the series of human embryos were reconstructed graphically, from tracings of each fifth section prepared with an Edinger apparatus at a magnification of 550 diameters. The reconstructions³ were stylized to the extent of being built along a straight central axis without regard to the slightly curving course of the duct (compare, for example, figs. 1C and 25).

OBSERVATIONS AND DISCUSSION

The entire endolymphatic appendage is, in its early development, at first a digitiform (figs. 2 to 5), and then a lanceolate (figs. 6 to 10) diverticular process of the otocyst. Very

² Only one series, the 74-mm., was more than 45 mm. in length, and was alone exceptional to the graduated arrangement of series.

³ These are reproduced in figures 2 to 25, and are the following: fig. 2, H.E.C., 1000; fig. 3, 2247; fig. 4, 2284; fig. 5, 2036; fig. 6, 2161; fig. 7, 2000; fig. 8, 839; fig. 9, 2051; fig. 10, 2155; fig. 11, 2246; fig. 12, 819; fig. 13, 1597; fig. 14, 871; fig. 15, 2048; fig. 16, 2046; fig. 17, 2045; fig. 18, 2042; fig. 19, 2248; fig. 20, 913; fig. 21, 2043; fig. 22, 2050; fig. 23, 820; fig. 24, 841; fig. 25, 2128.

soon after the whole appendage becomes differentiated into the commonly recognized subdivisions, ductus and saccus, a third segment appears, in the form of a narrow, distally placed, prolongation. This third subdivision, the distal pro-

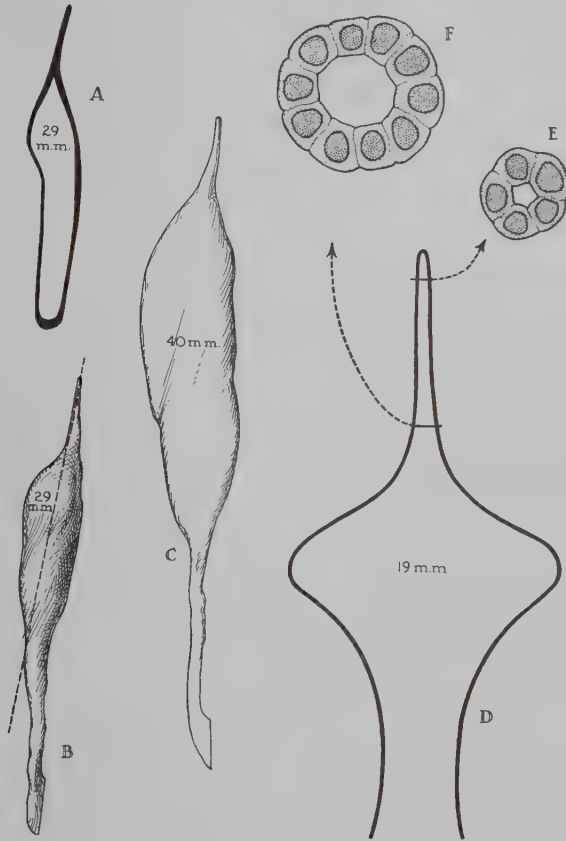
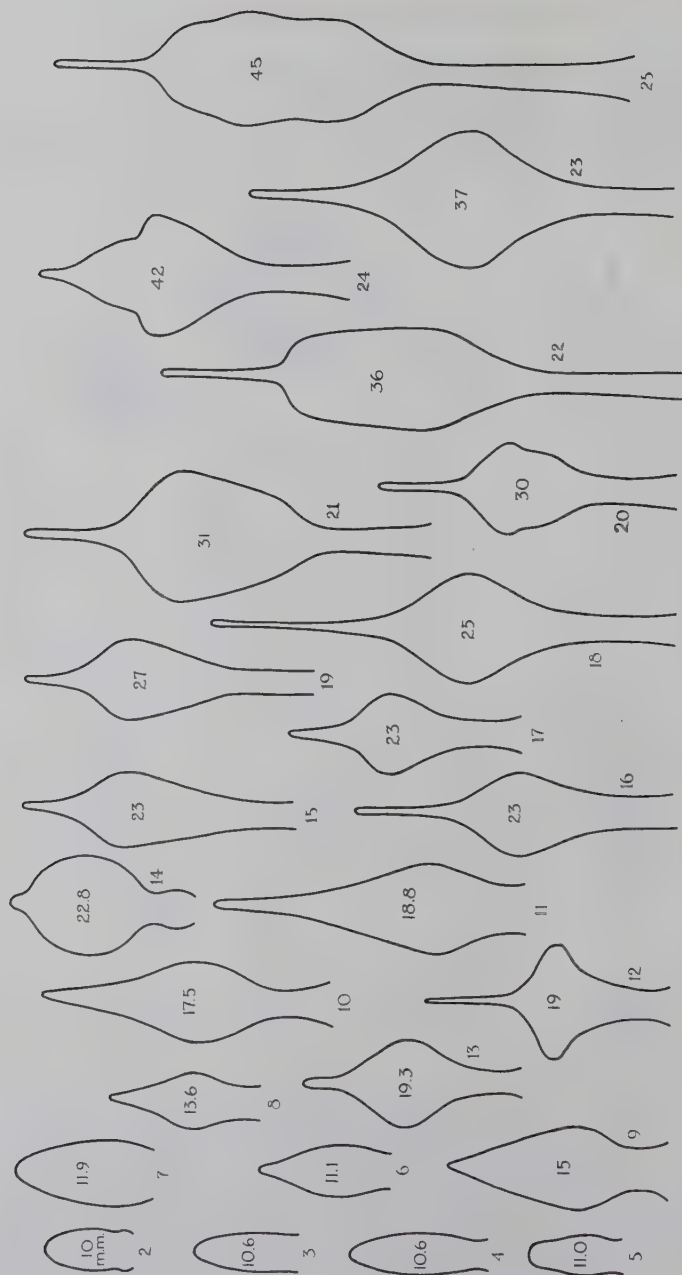


Fig. 1 Endolymphatic duct, sac, and distal prolongation of the latter, from human embryos in the Harvard Embryological Collection.

A. Tracing from a frontal section of a 29-mm. embryo (H. E. C., 914, sect. 392). $\times 30$. B. Model prepared from the same series. The level of figure A is indicated by broken line. $\times 30$. C. Model from a 40-mm. embryo (H. E. C., 1917). $\times 30$. D. Reconstruction, from a 19-mm. embryo (H. E. C., 819; see also fig. 12). Levels of sections shown in the two succeeding figures are indicated. $\times 67$. E and F. Drawings (camera-lucida) at two levels (sections 87, 103) of the distal projection seen in figure D. $\times 760$.



Figs. 2 to 25 Graphic reconstructions of the right endolymphatic sac and duct from transverse series of human embryos.
(Harvard Embryological Collection.) Lateral views. $\times 27$.

jection, is unmistakably present in the embryo of 19 mm. (fig. 12; also 11 and 13), and seems to owe its formation not only to elongation of the terminal third and the primitive appendage, but also to accompanying constriction of this part. Then, while retaining its typical shape, further extension occurs through the 27-mm. stage (figs. 15 to 18)—the length of the entire endolymphatic appendage being tripled or quadrupled while its greatest width is but doubled. Thereafter, as shown by the most advanced stages included in our reconstructions (31-, 36-, 37-, and 45-mm. embryos; figs. 21, 22, 23, and 25), the distal projection retains its long thin form, while the saccus becomes more bulbous; the projection is still present in most advanced series available, the 74-mm. embryo. Concurrently, with the alterations described above, the ductus becomes, in general, progressively more elongate.

The development of the projection is not, in the successive embryos, invariably progressive—there occur exceptional forms and sizes. Thus in the 19-mm. embryo the projection (fig. 12) is in an advanced stage of development (a condition which is duplicated in the other labyrinth of the same specimen); in the 25-mm. embryo (fig. 18) there is a striking development of the appendage, this structure attaining a length one and a half times that of the endolymphatic duct.⁴ (Contrariwise, the outgrowth is occasionally poorly developed; in the 22.8-mm. embryo (fig. 14) it is merely nodular, and in an older embryo, one of 42 mm. (fig. 24), its basal portion forms a relatively wide bulging.

When seen in section (fig. 1 A), or when modeled (figs. 1 B and 1 C), the configuration of the projection is in substantial agreement with that displayed by the reconstructions.

Histologically, the wall of the distal projection, like that of the saccus and of the ductus, consists of a single layer of

⁴In this series the writer first encountered the structure which he then believed might represent the original stalk of communication between the outer ectoderm and the endolymphatic sac drawn out into attenuate form. Further study of series, as herein figured, proved that such was not the case. The history of the rupture of the connection with the ectoderm will be discussed in a subsequent publication.

cuboidal epithelial cells. In the region where the sac definitely narrows to become the dorsal extension, it is approximately ten cells in circumference (fig. 1 F); the number of cells gradually decreases to four or five in the terminal fourth, and the lumen becomes progressively smaller (fig. 1 E); the latter usually disappears in the distal reaches, so that the tip of the process is a solid cord, composed, in transverse section, of four, three, or even fewer cells.

The distal prolongation of the endolymphatic sac is, so far as our observations indicate, restricted in its occurrence to the embryo of man; it was not seen in the embryos of the other ten mammalian species included in this study.

A further study of this striking prolongation of the saccus endolymphaticus is now in progress. Although its fate in the fetal or the adult ear is as yet undetermined, it may be suggested that the structure is perhaps represented by the very irregular, or occasionally even contorted, termination of the endolymphatic duct observed in serial section of ears from some postnatal specimens.

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GLOMERULAR DEGENERATION IN THE KIDNEY OF THE DADDY SCULPIN (MYOXOCEPHALUS SCORPIUS)

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TWELVE TEXT FIGURES AND THREE PLATES (THIRTY-FIVE FIGURES)

The kidney of the common sculpin, *Myoxocephalus octodecimspinosus*, contains large numbers of glomeruli (Nash, '31) which show a considerable amount of function (Marshall and Grafflin, '32). It was surprising to find, therefore, that a 1-kg. specimen of the closely related species, *M. scorpius*, satisfied physiological criteria for the aglomerular condition—namely, failure to eliminate the following three substances: 1) glucose with a high plasma level (even when phlorizin was administered), 2) ferrocyanide, and 3) cyanol, each in appropriate dosage (Marshall and Grafflin, '32). Although yielding no evidence of glomerular function, the kidney of this specimen exhibited numerous glomerular structures; hence it became imperative to reconcile the anatomical with the physiological findings. The fact that some glomerular function could be demonstrated in other specimens¹ made it advisable to study the glomeruli in a series of daddy sculpins of different ages, and this has been done for sixteen animals,

¹ No. 5—475 gm. Urine/Plasma xylose: 0.14 urine flow: 11.7 cc. kg. 24 hours.

No. 6—525 gm. U/P. xylose: 0.46 urine flow: 4.7 cc.

The common sculpin has been shown to have a U/P ratio for glucose under phlorizin as high as 2.75 (Marshall and Grafflin, '32). The experimental basis for the use of xylose as a measure of glomerular filtrate has been supplied by Dr. Homer Smith and his co-workers (Jolliffe, Shannon, and Smith, '32; Clarke and Smith, '32).

varying in weight from 85 to 1006 gm.² Blocks were taken routinely from the posterior portion of the kidney, but in some specimens representative blocks were taken also from the anterior and middle portions.

CONDITION OF GLOMERULI WITH REFERENCE TO WEIGHT OF FISH

In the kidneys of the three youngest fishes (85 to 125 gm.) marked evidences of glomerular degeneration are already present. There are large numbers of small, shrunken glomeruli with completely avascular tufts. These are connected with tubules by markedly constricted neck segments, the lumen of which is in many cases still patent; in others it is represented only by a fine, glairy channel, obviously non-patent; and in still others it is completely obliterated. At the other extreme are found rather large, markedly lobulated glomeruli showing excellent vascularization, a very delicate glomerular membrane, and tubular outlets which are of good size and entirely normal in appearance. From all anatomical considerations the large glomeruli of the latter variety must be considered functionally competent and as affording a sound basis for any glomerular function demonstrated by physiological tests.

Between the very good and the very poor glomeruli are found all gradations, in some of which glomerular function must be considered quite possible, in others doubtful, and in still others inconceivable. The degenerative changes affect both the tufts and the neck segments. Many large or medium-sized tufts show a definite loss of vascularity and concomitantly a considerable increase in the cellular make-up of the tuft, which may be generalized but as a rule predominates in the central portions. The extreme case is illustrated by figure 35. Many glomeruli, of different sizes and exhibiting various degrees of degeneration, show mild to moderate increase in the size of the intracapsular space, associated or not,

² These fish were collected at the Mount Desert Island Biological Laboratory, Salisbury Cove, Maine. The young specimens were further identified by counting their pyloric appendages, which averaged around nine, characteristic of *M. scorpius*, whereas for *M. octodecimspinosus* the average is around four.

as the case may be, with intracapsular coagulum, but invariably associated with marked constriction of the tubular outlet. Some glomeruli show central cystic degeneration and a few show degeneration of a caseous type (see below under 'Glomerular types').

The renal tubules subsequent to the constricted neck segments have degenerated in relatively few instances. These few tubules show, however, all degrees of degeneration. Some of them are of fair size (fig. 1; also fig. 26) and only moderately basophilic (even slightly eosinophilic in some parts of the tubule); others are small, shrunken and markedly basophilic throughout (fig. 2; also figs. 24 and 25), with all stages between these two extremes.³ From a study of the characteristics of the normal epithelium the basophilia must be interpreted as a sign of degenerative change. In addition some short degenerative tubules of the type shown in figure 12 are present, and the isolated instance of tubular degeneration illustrated in figure 11 comes from one of these kidneys.

Passing at once from these young specimens to one of the oldest (no. 92—1000 gm.—functionally aglomerular), we find the degenerative processes markedly advanced. The great majority of the glomeruli show such advanced changes in the tuft and in the neck segment that they can be ruled out at once as incapable of functioning. Accordingly, a search was made for the best-looking glomeruli, which of all those present were most likely to be functional. The selection of these glomeruli was contingent upon their meeting three simple criteria: 1) the presence of normal red cells in the capillaries (to insure patency of the afferent vessel); 2) the lack of intracapsular coagulum; and, 3) the lack of distention of the intracapsular space. Selected on this basis, thirty-two glomeruli were culled from many sets of serial sections and then studied in detail. It must be emphasized, however, that

³ In addition to these rather characteristic degenerate tubules, one sometimes finds a hyaline, granular type of tubular degeneration. I omit this from the discussion here because I have found it in other teleostean kidneys which are entirely devoid of the characteristic degenerative changes which are the subject of this study.

although these were the best glomerular tufts to be found in the material, they were nevertheless quite poor. Decrease in delicacy of the glomerular membrane and lobulation, and increase in cellularity were often so marked that, on anatomical grounds, the tufts could hardly be considered capable of anything approaching normal function. In every instance

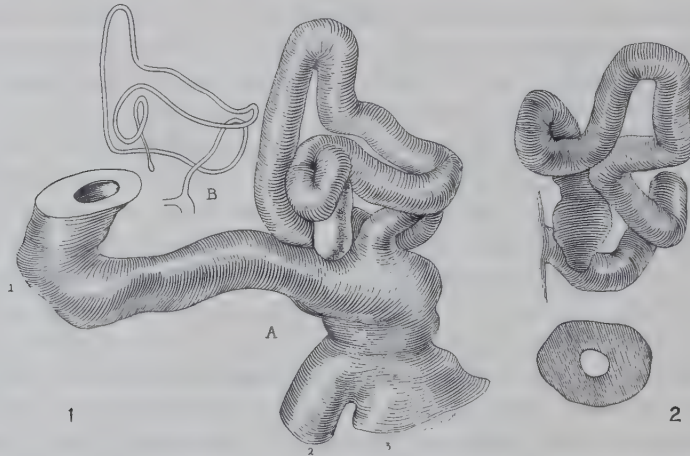


Fig.1 Drawing from wax reconstruction of a nephron showing degenerative changes throughout, but at an earlier stage than is illustrated in figure 2. B is merely a wire drawing to show the convolution of the degenerate tubule in A. Limb I is the beginning of a normal renal tubule; limb 2 on tracing passes shortly into another normal renal tubule. Limb 3 is passing off at an angle and soon connects with a large branch of the collecting duct system. $\times 265$.

Fig.2 A very degenerate nephron, small and markedly basophilic throughout. Total length of nephron is 0.47 mm. To right is shown cross section of normal renal tubule for comparison of size. Wax reconstruction. $\times 265$.

there was marked constriction in the neck region, and in only eight instances, or 25 per cent of the cases, could the neck lumen be considered anatomically patent, with functional patency very questionable. In the remaining twenty-four, or 75 per cent of the total, it was impossible to conceive of anatomical patency in the neck lumen. In thirteen instances there was complete obliteration of the lumen at some point in the neck segment, not even the barest outlines of the original

lumen being discernible. In one instance separation of the capsule from the tubule was complete. In still another instance the capsule tapered off gradually into a small tubular segment, lined with low cuboidal epithelium, which finally ended blindly (figs. 8 and 23).⁴

In a maceration study of this kidney, the neck regions were constricted and peculiar in appearance, and lumina were often exceedingly difficult to make out. Many glomerular

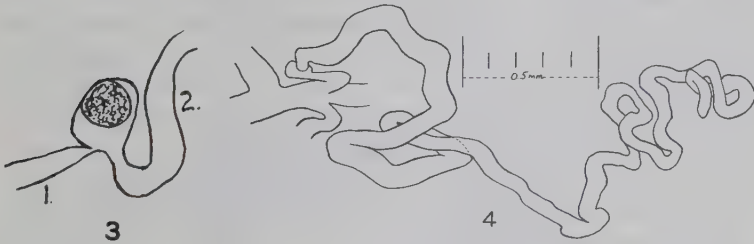


Fig. 3 A glomerulus showing two tubular connections, isolated from maceration material of no. 92. Tubule 1 was traced to its connection with a collecting duct; tubule 2 had been broken at some distance from the glomerulus. $\times 100$.

Fig. 4 A bona fide aglomerular nephron teased from maceration material of no. 92 (functionally aglomerular). $\times 40$.

capsules were very abnormal in shape, suggesting at once the conditions found in the goosfish (Grafflin, '29). Two instances were found of a single glomerulus connected with two renal tubules (fig. 3), and I was subsequently able to find one instance of this condition in sectioned material. Also I was able to tease out one bona fide aglomerular nephron (fig. 4).

Thus the paradox of a kidney showing many glomeruli anatomically but no glomerular function physiologically has been satisfactorily explained by this study. The glomeruli have been rendered incompetent by degenerative changes af-

⁴In view of the small size and delicacy of the structures involved, I obtained a series at 3μ for comparative study. The conclusions to be drawn from these sections confirm in all essentials those drawn from the 5μ series. The ratio of patent to non-patent necks is about the same and the pictures check in all details. From a study of many 3μ series in the course of this investigation I am convinced that, for this material, the findings in the readily obtainable 5μ series can be interpreted except in rare instances with complete confidence.

fecting both the vascular tufts and the neck segments, so that it is practically impossible to find a single glomerulus which on anatomical grounds can be considered functional.

The kidneys of these four animals sound the keynote for the findings in the entire series, and it is unnecessary to give a detailed description of each of the other twelve fish. Essentially as we pass to specimens of greater and greater weight, the anatomical basis for an appreciable normal glomerular function becomes less and less satisfactory. I do not wish, however, to give the impression that there is an uninterrupted sequence of changes as we pass to sculpins of increasing weight, so that it is possible to predict the weight of a given fish with accuracy from the glomerular status. Animals of similar weight often vary considerably in regard to the degree of degenerative changes. Nevertheless, in a broad sense the fish show progressively greater glomerular changes with increasing age (as judged by weight).

GLOMERULAR TYPES

While the general character of many of the glomeruli found in this material has already been indicated, some of the glomerular types must be described in more detail. Glomeruli showing a mild or moderate degree of capsular distention are rather common, varying markedly in extent and number in different kidneys (for very marked distention see figs. 9, 13, and 18). The distention may be fairly uniform in all directions, but not infrequently takes the form of a marked elongation of the capsule, as shown in figure 5. Intracapsular coagulum is highly variable in its occurrence and, in the general run of glomeruli showing it, it is present in lesser amounts than we see in this figure.

The average small avascular tuft, to which reference has been frequently made, is shown in figure 28. In such a tuft the nuclei stain rather uniformly; but study of large numbers of these glomeruli has demonstrated the frequent occurrence of a fairly distinctive type in which the peripheral nuclei stain darkly while the central nuclei stain very poorly indeed (fig.

6). In a third type, presumably arising from the further atrophy and degeneration of a structure such as is shown in figure 6, the glomerular tuft is represented by only a few small cells with scanty cytoplasm (fig. 34).

Two further types, in addition to being avascular, are acellular. In one the tuft is represented by a greenish (in H. and E.) and rather caseous looking mass which may preserve the outlines of the original glomerular structure. In some instances the afferent vessel can be observed to have undergone similar degeneration and on tracing disappears in the nearby interstitial tissue (fig. 32). The second acellu-

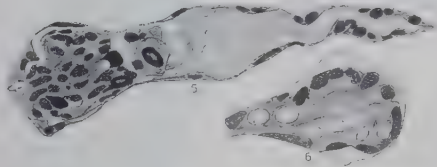


Fig. 5 A glomerulus with an elongated capsule. Though the tuft is markedly cellular some of the capillaries are still patent and contain normal red cells (in black). The capsular membrane is thickened in some regions, particularly in the tapered extremity, which connects with a renal tubule whose lumen is completely obliterated in the neck segment. There is a fairly delicate coagulum filling most of the intracapsular space. Camera lucida. $\times 540$.

Fig. 6 An avascular tuft with two types of nuclear staining. Camera lucida. $\times 830$.

lar type may be designated as a 'shell' glomerulus. Here the original tuft is represented merely by glairy, more or less well-defined membranes in which practically all semblance of structure has been lost. This type may presumably be interpreted as a later and more degenerate stage of a tuft such as is shown in figure 31. Its general arrangement and attachment to the capsule at the point of entrance of the original afferent vessel rule out the possibility of its being non-glomerular in origin, e.g., arising from a parasitic structure of Group II (see below).

Probably the most interesting glomeruli in this material are those showing what may be called a central cystic degen-

eration. In some instances the tuft shows a more or less spherical and very well-delimited cavity (fig. 27) which is either entirely free from coagulum or shows it in only small amounts. These clear spaces show a wide variation in size, many of them being so small that they are visible in only one 5μ section of a series. The sharp delimitation of these central cysts in some cases suggests that we may even be dealing here with a malformation of the tuft. The peripheral rim of tissue shows a variable increase in cellularity. In other tufts

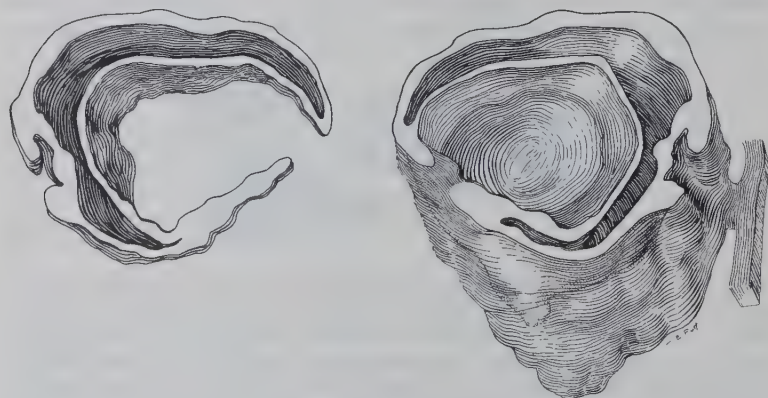


Fig. 7 Drawing from wax model of a striking instance of central cystic degeneration with dense coagulum. The upper portion of the model has been removed and is shown inverted to the left. The dotted area in the main figure indicates the extent of the central lake of coagulum. To the right are indicated the entrance of the afferent vessel and the point of reflection of the Bowman's capsule. $\times 350$.

the cyst shows rather poor delimitation and considerable amounts of coagulum (fig. 30). In figure 7 is illustrated a wax model of one very striking instance of central degeneration. The peripheral rim of tissue shows fair preservation of glomerular structure, the entire center is occupied by a lake of dense coagulum, and the tuft has apparently collapsed on one side, to give much the same picture as if one exerted pressure with his finger upon a hollow rubber ball (fig. 15). The Bowman's capsule, which is entirely free from intracapsular coagulum, shows an irregular bulbous out-pocketing

in which was found a parasitic structure belonging to Group II (see below). No tubular connection with this capsule could be found. In figures 29 and 31 are illustrated two tufts in which the central degeneration has progressed so far that there remains only a thin rim of tissue in which some slight semblance of the original glomerular structure is preserved, and in which patent capillaries, containing normal red cells, are present.

There remains to be mentioned a very unique instance in which the original tuft is represented by a shadowy structure with occasional nuclei and nuclear fragments scattered here and there through its structure (fig. 37). While this tuft is suggestive of the conditions found in the central portion of many glomeruli in the goosefish (Grafflin, '29, fig. 5), it is interesting that in this entire material I have been unable to find a single glomerulus which duplicates those conditions.

DISCONTINUITY OF NEPHRONS

We have already seen how frequently functional and anatomical continuity of the nephron is broken by obliteration of the lumen in the neck segment. Instances have been found in which the separation of capsule from tubule is complete, and where the nearby aglomerular tubule, normal except at its degenerate tip, can nevertheless be related to the isolated glomerulus with perfect confidence. In figures 8, 9, and 10 are shown three instances of discontinuity at a short distance from the glomerulus. The tapering portion of figure 8 is lined by a low cuboidal epithelium, tubular rather than capsular in nature. The tuft, well vascularized but lacking in delicacy (fig. 23), was one of the best to be found in the kidney of no. 92 (functionally aglomerular). The structure in figure 9 was naturally singled out for study because of the enormous capsular distention (fig. 13). The tuft, though quite cellular, retains some patent capillaries. The lumen is completely obliterated in the neck segment but is patent in the small tubular stump which is attached (see figs. 14, 16, and 17 for details). The structure in figure 10 shows discontinuity at

about the same point in the tubule but in this instance capsular distention is lacking and the lumen in the neck segment is fully patent. The glomerular tuft is quite cellular, but shows peripherally a few patent capillary loops (fig. 19).

Figure 11 illustrates an instance of separation at some distance from a glomerulus, the tuft of which is well vascularized though lacking in delicacy (fig. 20). The tubular segment

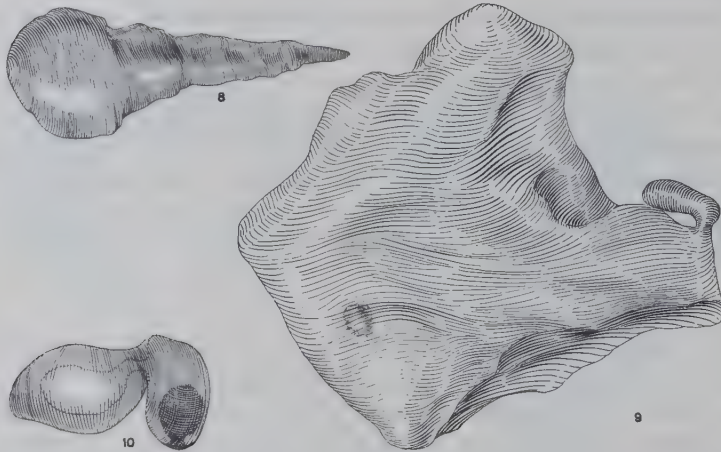


Fig. 8 A case of pinching off of the tubule very close to the glomerulus. See description in text; also see figure 23. Drawn from wax reconstruction. $\times 250$.

Fig. 9 A very striking instance of enormous capsular distention. The glomerular tuft is stippled. See description in text; also see figures 13, 14, 16, and 17. Drawn from wax reconstruction. $\times 125$.

Fig. 10 A curious instance of a glomerulus connected with a small bulbous tubular segment. See description in text; also see figure 19. Drawn from wax reconstruction. $\times 250$.

attached to the glomerulus is degenerate throughout and ends blindly; but a short distance from its ending an aglomerular nephron starts which is quite long, perfectly normal histologically, and undoubtedly originally connected with the neighboring degenerate tubule. The continuity is actually still indicated by strands of rather basophilic connective tissue. In figure 12 is illustrated one of many small, blind, basophilic tubules found in connection with branches of the collecting

duct system. The exact mode of origin of these degenerate tubules is not indicated in my material.

PARASITISM

The kidneys of the daddy sculpin have been found to be rather extensively invaded by two groups of parasites. In view of the nature of certain tissue reactions evoked by the

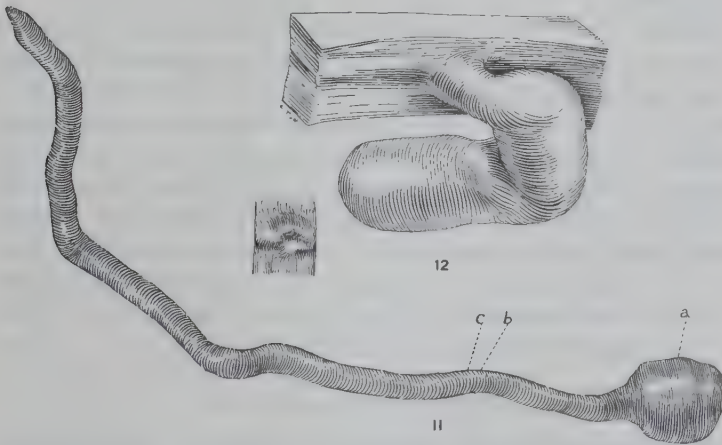


Fig. 11 Showing a glomerulus and tubular segment which have separated completely from the more distal part of the tubule. See description in text; also see figures 20, 21, and 22 for microphotographs at levels a, b, and c. Drawn from wax reconstruction. $\times 325$.

Fig. 12 A small, degenerate, basophilic tubule, ending blindly and connecting with a fairly large branch of the collecting duct system. The lumen is patent and coagulum free except in the constricted portion as it passes through the wall of the duct. The size of the opening is shown below in the small sketch. See description in text. Drawn from wax reconstruction. $\times 540$.

group II parasites, suggesting proliferative changes in a glomerulus (fig. 47), a thorough study was made to determine whether the parasitism could in any sense be regarded as a causative factor in the glomerular and tubular degeneration occurring in these kidneys. The conclusion is drawn that it cannot possibly be so considered.

Group I. In every kidney of this series, there occurs in large numbers, a small unicellular structure which I can only interpret as a form of parasite, but which I have been completely unable to identify (figs. 38 to 41). These parasites apparently occur indiscriminately in the epithelium of the various portions of the collecting duct system and of the nephron, with the exception of the neck segment and the glomerulus.

Group II. This group includes a wide variety of multicellular structures (figs. 42 to 47) which can nevertheless be most plausibly interpreted as arising from invasion by a single parasitic form. These parasites are in no way similar to those of group I, and I am convinced that there is no relationship between the two groups. While all the kidneys show group I forms in large numbers, many kidneys are entirely free from those of group II.⁵ With two exceptions, when they were located in the intracapsular space of a glomerulus (fig. 44; text description of fig. 9), the structures have been found in the interstitial tissues of the kidney.

DISCUSSION AND SUMMARY

Defrise ('32) has reported, from a study of the character of the Golgi substance, that the aglomerular nephron of the toadfish consists of only one segment (Golgi in the form of spherical masses) whereas the glomerular nephron of the common sculpin consists of two segments (Golgi rods in the proximal portion, Golgi masses in the distal),⁶ both of which, however, show brush border. From Defrise's findings the conclusion might easily be drawn that the distal segment of sculpin nephron is directly comparable with the single secre-

⁵ The incidence of group II parasites is as follows: five specimens, 185 gm. and below, are uniformly free from parasites; of four specimens between 300 and 500 gm., two show parasites (in small numbers) and two do not; seven specimens between 500 and 1006 gm. are all infested to a variable extent. It so happens that the heaviest fish (1006 gm.) shows the most extensive infestation in the entire series, while no. 92 (1000 gm.) shows the parasites in rather small numbers.

⁶ In Defrise's paper there is an error in the plate legends bearing on this point, but the statement in his text is correct.

tory segment of the toadfish, and that the proximal portion in the sculpin is concerned solely with reabsorption from the glomerular filtrate.⁷ If this were true, we should expect this proximal segment to degenerate following the obliteration of the associated glomerulus. However, in the present study of hundreds of glomeruli in which the entire vascular bed is obliterated, the neck segment is the only portion of the associated tubule which shows collapse and degeneration (disregarding the relatively few instances in which the entire nephron degenerates). The persistence of the proximal segment under these conditions is interpreted as excellent evidence that this segment normally carries out some function other than, or in addition to, reabsorption from the glomerular filtrate. However, one cannot rule out the possibility that this segment is normally devoted entirely to reabsorption, but, with cessation of function in the glomerulus, changes its reabsorptive function to one of tubular elimination.

It is concluded from this study that in marine teleosts in general, results obtained by injecting a colored mass into the vascular system for the demonstration of the total number of active glomeruli must be interpreted with the greatest caution. In the daddy sculpin such results would be valueless because many glomeruli with no outlet to the bladder show complete patency of the vascular bed, as indicated by the presence of normal red cells in the tuft capillaries.

The paradox, in an old specimen of daddy sculpin, of a kidney showing many glomeruli anatomically but no glomerular function physiologically has been satisfactorily explained by this study; and confidence in the adequacy of our

⁷ Careful cytological studies have not been carried out on the daddy sculpin kidney, and the following discussion is based on the assumption that the two segments as defined by Defrise for the common sculpin are also present in the daddy sculpin. On histological grounds (granular content, degree of eosinophilia) I can demonstrate quite readily in some kidneys of this series two distinct segments, both of which show brush border. The transition from the first to the second segment is very abrupt in the many tubules in which I have studied it. This should be noted because Defrise found a rather long intermediate transitional segment in the common sculpin (personal communication to Doctor Marshall).

physiological criteria of the aglomerular condition is thereby strengthened. The glomeruli have been rendered incompetent by degenerative changes affecting both the vascular tufts and the neck segments, so that it is practically impossible to find a single glomerulus which on anatomical grounds can be considered functional. In young fish of the same species there can be found adequate anatomical basis for the varying, but low, glomerular function which can be demonstrated physiologically. However, considerable degeneration is already present in the youngest specimens examined, and these changes become steadily more prominent with increasing age.

The persistent oliguria in marine, relative to fresh water, teleosts has been held by Smith ('29) and Marshall and Smith ('30) to be related to the glomerular degeneration occurring in the marine forms. In the daddy sculpin we are dealing with an animal in which this glomerular degeneration proceeds during the life of a single individual to such an extent that various stages of degeneration can be observed in fishes of different ages. This degeneration is interpreted as a physiological involution rather than as a pathological process. There is no evidence that two types of parasites occurring in these kidneys play any role in causing the observed degenerative changes.

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PLATE 1

EXPLANATION OF FIGURES

The figures on this plate, with the exception of figure 18, are documentary for wax reconstructions illustrated in the text. All sections are at 5μ and are stained with H. and E., with the exception of figure 15 (Mallory's connective tissue stain).

Figures 13, 14, 16, and 17 are documentary for the reconstruction illustrated in figure 9.

13 Low power photograph, showing enormous capsular distention. Note the glomerular tuft below and the relatively small amount of coagulum. $\times 185$.

14 Showing the neck leading off from the Bowman's capsule. $\times 450$.

15 Cross section of the glomerular tuft illustrated in figure 7. The clear central area corresponds to the depression in the tuft as shown in figure 7. Note the heavy coagulum and the fair preservation of glomerular structure in some portions. $\times 335$.

16 Showing the character of the tubule near its connection with the glomerular capsule. Note the group of dark nuclei arranged around the small, irregular, coagulum-filled lumen. $\times 450$.

17 A section through the long axis of the small, blind tubular nubbin. Note the 'shaggy' cell borders. Compare with normal tubule in figure 22 at same magnification.

18 A second instance of tremendous capsular distension. The glomerular tuft can be seen surrounded by large numbers of red blood cells. The capsule was damaged in the original cutting of the block. $\times 155$.

19 Section through the structure illustrated in figure 10. See description in text. $\times 480$.

Figures 20, 21, and 22 document the reconstruction shown in figure 11.

20 At level a. Note increase in cellularity of tuft and lack of delicacy of glomerular membrane. $\times 450$.

21 At level b. $\times 450$.

22 At level c. This cuboidal type of epithelium persists to the end of the degenerate segment. Note the normal renal tubule below for comparison. $\times 450$.

23 Section through the glomerular tuft of the structure in figure 8. $\times 480$.

24 Section through the glomerulus, at its greatest extent, of the degenerate tubule shown in figure 2. $\times 480$.

25 Section through the degenerate tubule shown in figure 2, at the level of its connection with a large collecting duct. Below to the left is the point of connection, the lumen being closed off by a thin membrane. Just above is the markedly constricted neck segment with a pin-point lumen, at a short distance from the glomerulus. The basophilia of the tubule is quite evident. $\times 480$.

26 Section through the degenerate tubule illustrated in figure 3, at the level of the glomerulus, which is completely cellular. In the lower right-hand corner is a section of the normal renal tubule 1 of figure 1. $\times 400$.

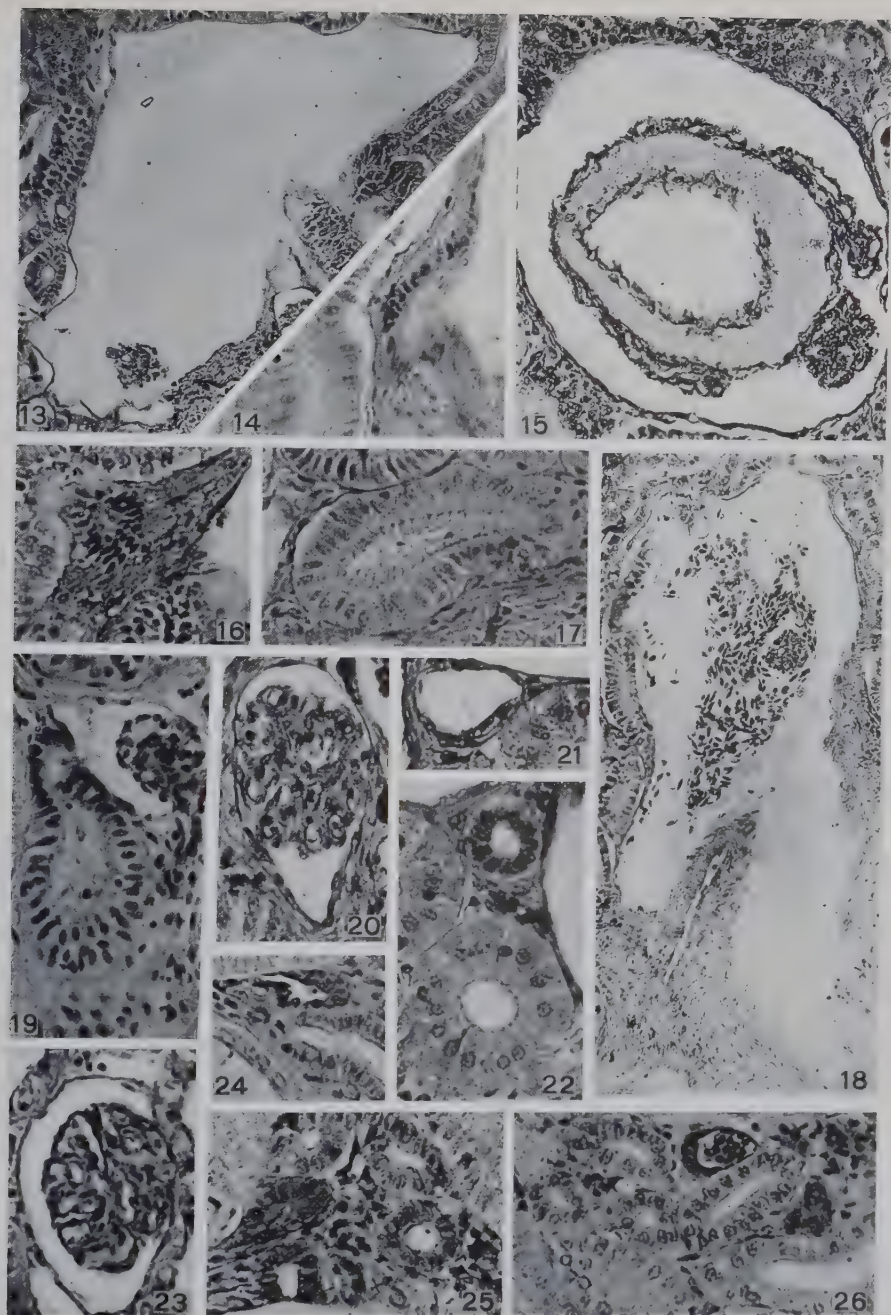


PLATE 2

EXPLANATION OF FIGURES

27 A tuft showing a sharply delimited central cyst with only small traces of coagulum. 5μ . $\times 680$.

28 A small, average avascular tuft. 5μ . $\times 480$.

29 A tuft showing marked central cystic degeneration with heavy coagulum. The thin peripheral rim of tissue is well preserved and shows a few patent capillaries. 5μ . $\times 200$.

30 A tuft showing central cystic degeneration with fairly light coagulum. The central cavity is not well demarcated as compared with the type illustrated in figure 27. 5μ . $\times 480$.

31 A tuft showing marked central cystic degeneration with heavy coagulum. There are many patent capillaries in the peripheral rim of tissue. 5μ . $\times 680$.

32 A tuft showing a rather caseous type of degeneration in one portion with fair preservation of the remainder. The outlines of the obliterated afferent vessel may be seen in the lower right. The good portion of the tuft derives its blood supply from secondary connections between the tuft and the capsule. 5μ . $\times 480$.

33 A very striking instance of capsular distension and intracapsular coagulum. The tuft is quite cellular but still shows patent capillaries. The tubular outlet could not be considered patent. 5μ . $\times 255$.

34 One of the atrophic, avascular tufts represented by only a few cells with scanty cytoplasm. 3μ . $\times 1530$.

35 A tuft showing good vascularity peripherally, but complete cellularity in the central region. 5μ . $\times 480$.

36 A so-called 'shell' glomerulus, showing complete obliteration of the original structure of the tuft. The intracapsular space shows no trace of coagulum. 5μ . $\times 480$.

37 A unique tuft showing a delicate, shadowy structure with the persistence here and there of nuclei and nuclear fragments. 5μ . $\times 370$.

38 A portion of the wall of a main branch of the collecting duct system showing large numbers of group I parasites. 5μ . $\times 480$.

Figures 27 through 38, H. and E.; figures 39 through 41, Heidenhain azan.

39 Two group I parasites in cross section at the level of the nucleus. 3μ . $\times 900$.

40 Two group I parasites. The capsule of the parasite to the left stained blue, and the nucleus shows areas of condensation. The capsule of the parasite to the right stained yellowish orange. The clear areas, indicating shrinkage, are a frequent finding. 3μ . $\times 900$.

41 Three group I parasites. The capsule in each instance stained yellowish orange, the nucleus red. 3μ . $\times 900$.

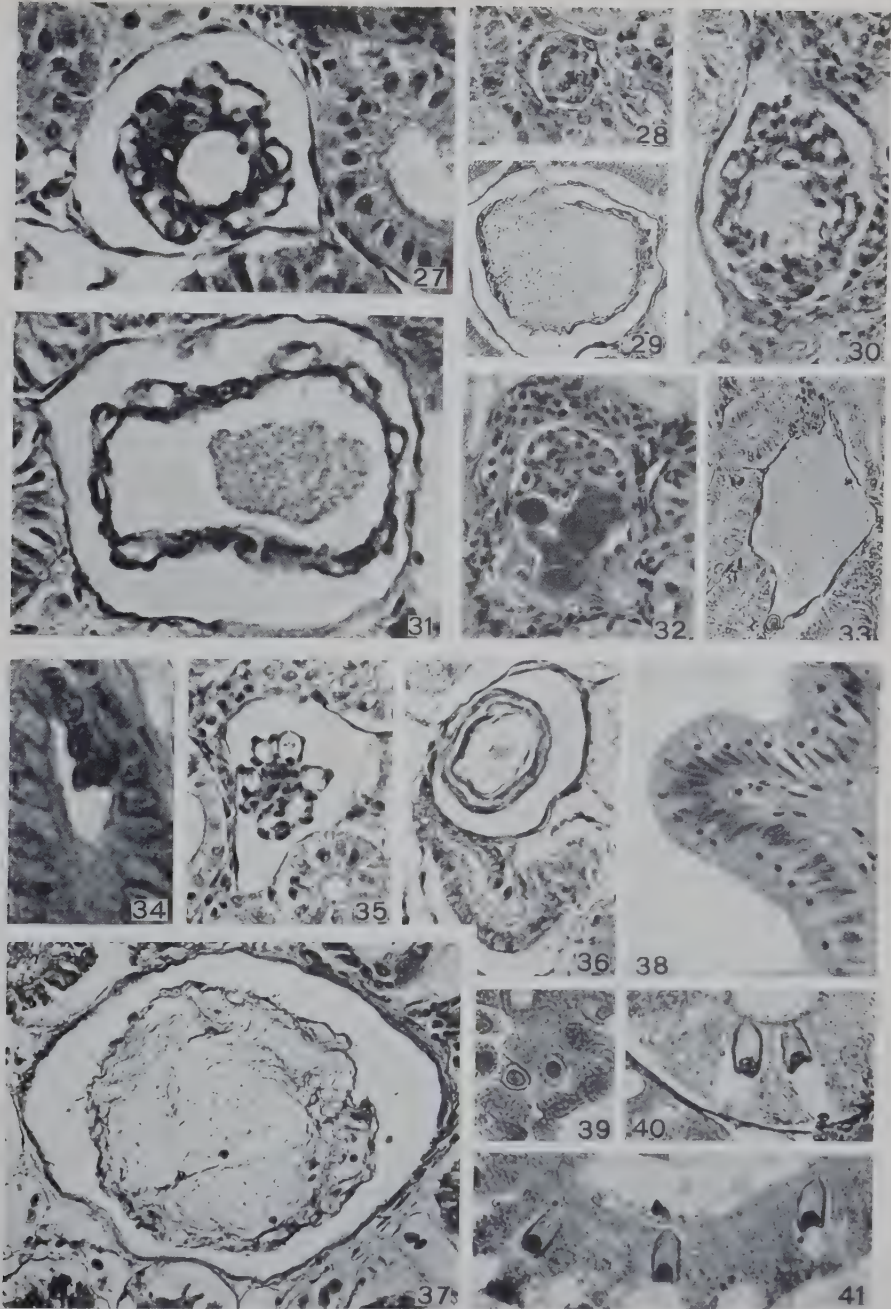


PLATE 3

EXPLANATION OF FIGURES

The figures in this plate illustrate various structures associated with the presence of group II parasites. All sections stained with H. and E.

42 This is one of the clearest examples in my material of what I take to be the most characteristic form of the parasite. An essentially similar organism can be seen in figure 3. In the tissue reaction about the parasite, in this instance, are many cells loaded with yellow pigment. The entire structure is surrounded by a capsule of dense connective tissue. 3μ . $\times 665$.

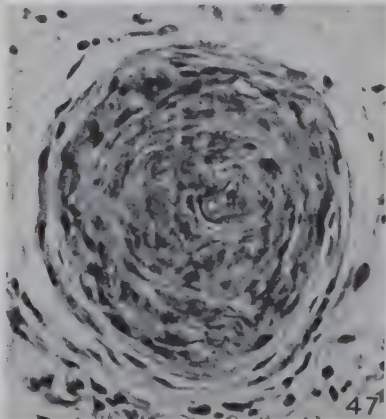
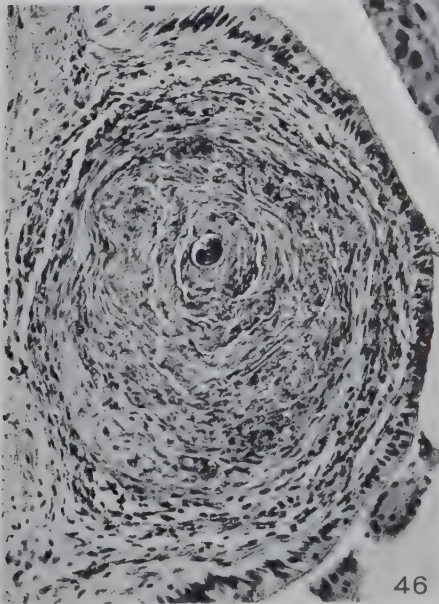
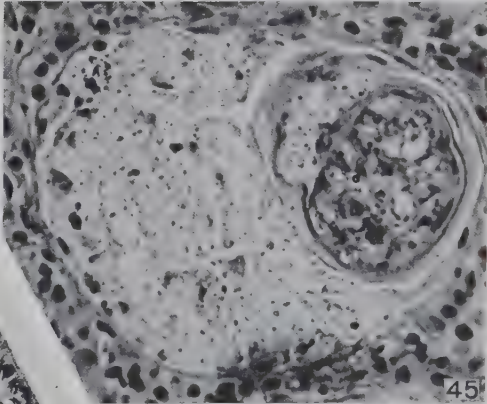
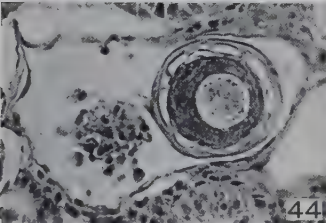
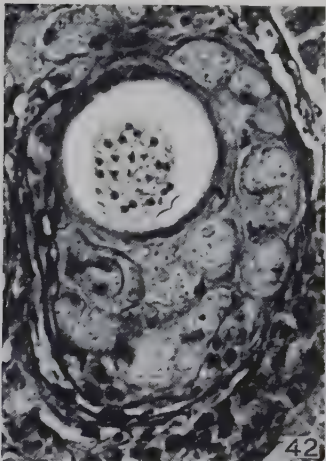
43 This is one of the forms which the parasite structures may take. The bulk of the structure is a rather formless, dense coagulum, with nuclear fragments scattered here and there through it. Near the center of the coagulum is a small region separated from the surroundings by a rather delicate capsular membrane. The formless contents of this region are difficult to interpret, but I am led to assume that they represent the remains of the original parasite. Adjacent to the coagulum is a group of cells (rather clear in the photograph) which are loaded with yellow pigment. The entire structure is surrounded by a dense connective tissue capsule. The light area to the right of and below the capsule is a group of pale yellow pigment cells. 3μ . $\times 665$.

44 This is the one of two instances in which a parasitic structure has been found within a glomerular capsule. Note a portion of the glomerular tuft to the left of the parasite, also the coagulum throughout the intracapsular space. Although the nuclear masses are not as striking as in figure 1, the organisms are essentially identical but in different environment. 5μ . $\times 370$.

45 This structure is, I believe, fundamentally similar to that shown in figure 2. The denser portion to the right is made up of a rather homogeneous, eosinophilic ground substance with a large number of nuclei and nuclear fragments scattered throughout at random. Also here and there are small aggregates of yellow pigment. Above and to the left of this denser region is a lighter area containing a few cells loaded with bright yellow pigment. Surrounding the two is a rather dense connective tissue capsule. This portion of figure 4 I consider closely similar to the encapsulated portion of figure 2. The dense area with much nuclear material apparently represents the forerunner of a large mass of dense coagulum in figure 2. No discrete parasite or suggestion of a parasite (figs. 5 and 6) can be found in this multinucleated mass or elsewhere. If my interpretation is correct, the pale yellow pigment cells of figure 4 (the large, lighter, granular area) are analogous to the small groups of pale yellow to the right and below in figure 2. 3μ . $\times 665$.

46 The figure shows a very large concentric structure which I interpret as parasitic in origin, located in the wall of one of the main branches of the wolffian duct. Although I have no injected material, the entire center of these structures is certainly non-vascular. Near the center may be seen what is apparently all that remains of the original parasite. It was a well-defined, basophilic capsule containing some rather formless, eosinophilic material. This is an extreme instance of the marked cellular reaction which these parasites sometimes evoke in the tissues. 5μ . $\times 310$.

47 A smaller concentric structure located in the intertubular tissue. 5μ . $\times 665$.





GROWTH AND CELL PROLIFERATION IN HETERO- TOPIC SPINAL CORD GRAFTS

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FIFTEEN FIGURES

INTRODUCTION

That the chief stimuli affecting proliferation of cells within the spinal cord of larval *Amblystoma* reside within the central nervous system itself and not without, has been supported by many different experiments. Whereas peripheral overloading has been shown to bring about hyperplasias in the spinal ganglia, the cellular content of the cord secondarily brought into connection with a supernumerary peripheral structure, such as a grafted limb, has been shown to remain unaltered (Detwiler, '20 a, '23 a, '24). This failure on the part of the spinal cord to respond to volumetric alterations in the peripheral musculature led to a search for the nature of forces, apparently residing within the central nervous system, which affect cellular proliferation therein.

As a result of interchanging various spinal segments in the embryo, it has been possible to alter the cellular content of a given segment. The sixth, seventh, and eighth spinal segments, for example, which failed to undergo hyperplasia when in connection with a grafted limb, were found to proliferate more cells when grafted into the region normally occupied by the third, fourth, and fifth (brachial segments). This occurred regardless as to whether the limb was present or absent ('24). From these and other experiments involving reversal of various groups of segments ('23 b) it was suggested that one factor, which might be important in determining the extent

of cellular proliferation within the cord is the invasive growth of projection fibers from the medulla (bulbo-spinal fibers). In support of this thesis, experiments were carried out in which an additional medulla was grafted just caudal to the normal (Detwiler, '25). Since there resulted a hyperplasia of cells, particularly in the ventral regions of the segments just caudal to the interpolated medulla, it was suggested that this might be due to augmented stimulation associated with the additional projection fibers invading the cord.

Later, Nicholas ('28, '29, '30) permanently isolated the brain from the spinal cord by interposing a pronephros and limb just caudal to the medulla, following which a hypoplastic development of the cord occurred. These two types of experiments appeared to agree in demonstrating a definite stimulative role on the part of caudally growing projection fibers.

These and other experiments dealing with various interchanges of cord segments, and the interpretations which have been drawn from them, have been critically reviewed recently by Maclean ('32), to whose paper the reader is referred for a detailed survey.

In order to test further the supposed stimulative role of projection fibers upon proliferation of cells within the cord, Severinghaus ('30) carried out experiments in which units of isolated segments were grafted heterotopically to a region outside the cord. The third, fourth, and fifth segments with adjacent somites were grafted (with normal and reversed orientation) lateral to the third, fourth, and fifth somites of the host. The sixth, seventh, and eighth segments were grafted with similar conditions lateral to the corresponding host somites. These experiments were devised to study the effects of growth and proliferation of cells under conditions of total isolation of the segments from any stimulative influences which might normally arise from the central nervous system itself.

The results were as striking as they were unexpected in showing that the grafted segments not only grew to larger

size than the homologous intact segments of the host, but they proliferated cells considerably beyond the normal limits. While proliferation of cells in the isolated cord units was most excessive in the dorsal (sensory) regions, where increases of nearly 300 per cent over the normal were noted, hyperplasia in the ventral (motor) regions was obtained also (Severinghaus, *op. cit.*, fig. 4). Severinghaus discussed his results in relation to Coghill's ('24) studies upon localized regions of alternating waves of proliferation and differentiation within the amphibian nervous system, and pointed out further the obvious inference that normally fiber invasion secondarily brings a halt upon cell division, since, when the cells are removed from this influence, they continue to proliferate greatly in excess of the normal. He called attention to the possibility also that certain projection fibers may stimulate proliferation, whereas others may call forth differentiation. These two antagonistic influences acting upon the potential proliferative capacity at any given level of the cord would thus regulate the number of cells at various levels under normal conditions. Certain it is, from his results, that the proliferative potential of a given region of the cord is much greater than is normally expressed, and that there exists in the central nervous system the presence of some powerful restraining influence upon cell division, otherwise cellular proliferation in the isolated cord units would not have run riot, so to speak.

In a study of the development and segmentation of spinal ganglia, experiments were carried out in which heterotopic units of cords were grafted adjacent to the normal cord so that no muscle existed between the two (Detwiler, '32). In connection with these studies upon the growth of ganglia, it was noted incidentally that the heterotopic cords were smaller in their transverse dimensions than the normal—a condition in contrast with that found in the experiments of Severinghaus where the heterotopic cords were grafted lateral to the host's myotomes.

Since, in some instances, these isolated units of cord had no connection with the normal and were yet subnormally developed, it became apparent that some restraining influence other than that which might be associated with the growth of projection fibers was active. The present paper, therefore, deals with a comparison of the growth and proliferation of cells within heterotopic units of cord when grafted adjacent to the normal cord, and more distant as in the experiments of Severinghaus.

EXPERIMENTAL

In the first series of experiments the right sixth, seventh, and eighth somites were removed from a host embryo, as well as the corresponding left somites from a donor embryo. The donor cord along with the right somites was then grafted as a unit into the host wound so as to bring the sixth, seventh, and eighth segments of the two cords adjacent, as is illustrated in figure 1. Embryos of *A. punctatum* and *A. tigrinum* in stage ± 28 were employed in most of the operations, although in some, stage 23 was used so that the cord was grafted before the mesoderm had segmented. *Tigrinum* embryos constitute very desirable material for this type of experiment, since the somites can be excised with greater facility than in *punctatum* embryos.

The second series constitutes a repetition of the experiments of Severinghaus in which a cord with the mesial portion of the adjacent somites of both sides was grafted into a wound lateral to the host somites and beyond (see figs. 2 to 6 of Severinghaus and fig. 2 of this paper).

In both series of experiments the notochord was taken with the graft and, in the former series, the denuded side of each cord was well cleaned so that when graft and host cords were brought into contact no loose mesenchyme cells intervened.

The larvae were raised for varying lengths of time, ranging from 40 to 60 days after the operation. They were fixed in either warm Bouin or in vom Rath's fluid. Serial transverse sections 10μ in thickness were made of twenty-five cases of the first series and of ten cases of the latter series.

Comparisons of the size of the graft with the homologous region of the intact cord were made by graphic reconstruction, and by planimeter measurements of homologous cross section areas. An index of cellular proliferation at desired levels was obtained by estimating the mean number of cells per section in three alternate sections at homologous nerve levels passing through both graft and host cord. Dorsal

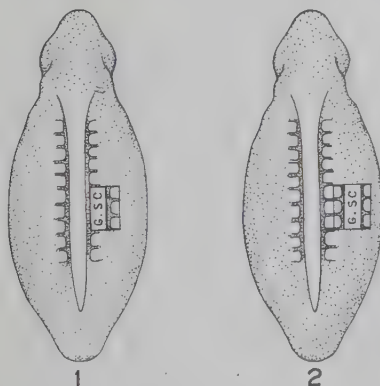


Fig. 1 *Amblystoma* embryo in stage $28\pm$, showing a piece of grafted cord (with right somites) adjacent to the normal cord. Before grafting, the right sixth, seventh, and eighth somites of the host embryo were excised, as were also the left sixth, seventh, and eighth somites of the donor embryo. *G-SC*, grafted spinal cord. $\times 10$.

Fig. 2 Embryo in stage $28\pm$, showing the sixth, seventh, and eighth segments of cord with adjacent mesial portions of the somites which were grafted as a unit into the lateral somitic region of the host on the level of the sixth, seventh, and eighth segments. $\times 10$.

(sensory) and ventral (motor) cellular regions were arbitrarily distinguished by bisecting the dorso-ventral axis of the cellular areas by a right angle line. All cells dorsal to the bisecting line were considered for purposes of comparison as sensory and those ventral to it as motor. This method which has been employed in former experiments, although not strictly accurate, does give a means of correlating proliferation in dorsal and ventral regions under various conditions.

Description of case—series 1

Case SCMT-16 (54 days, 40 mm.). The grafted cord was found to be smaller than the normal cord throughout its entire extent (table 1). Cross section areas of transverse sections from both cords are shown in figure 3, where it can be seen that through the sixth and seventh nerve levels the grafted cord is only 60 per cent as large as the normal (v. table 1).

TABLE 1

Ratios obtained from cross section areas and cell counts of selected sections through each nerve level (6, 7, 8) in both normal and grafted cords. The figures for the sixth, seventh, and eighth normal segments were divided, respectively, into those obtained from homologous grafted segments

CASE	AREA RATIOS			CELL RATIOS		
	6N: 6G	7N: 7G	8N: 8G	6N: 6G	7N: 7G	8N: 8G
A						
SCMT-16	1: 0.65	1: 0.63	1: 0.83	1: 1.21	1: 1.15	1: 1.03
SCMT-26	1: 0.65	1: 0.74	1: 0.52	1: 0.97	1: 1.14	1: 1.09
SCMT-17	1: 0.63	1: 0.66	1: 0.62	1: 0.97	1: 0.96	1: 1.37
SCMT-3	1: 0.62	1: 0.66	1: 0.65	1: 0.87	1: 0.90	1: 1.01
SCMT-1	1: 0.60	1: 0.83	1: 0.81	1: 0.88	1: 1.20	1: 1.02
B						
TrSCMPU-6	1: 1.50	1: 1.48	1: 1.40	1: 2.20	1: 2.03	1: 2.64
TrC-15	1: 1.44	1: 1.37	1: 1.15	1: 1.70	1: 1.75	1: 1.40
TrC5B	1: 1.29	1: 1.18	1: 1.09	1: 1.90	1: 1.57	1: 1.37
TrC-1	1: 1.89	1: 1.57	1: 1.20	1: 2.36	1: 1.70	1: 1.26
TrC-14	1: 1.72	1: 1.50	1: 1.33	1: 2.03	1: 1.40	1: 1.62
TrC5A	1: 1.49	1: 2.03	1: 2.50			
TrSCMT-3	1: 1.30	1: 1.60	1: 1.64			

A, grafted cord adjacent to normal.

B, grafted cord lies in lateral somitic region of host.

6N = Sixth normal segment.

6G = Sixth grafted segment.

The ratio of gray area to the total area in a given transverse section is slightly greater in the grafted than in the normal cord. Whereas the gray areas (as estimated by the planimeter) in the grafted cord are smaller than in the normal, the mean number of cells per section through homologous regions are slightly higher in the graft than in the normal. This shows, then, that, whereas the grafted cord has suffered a

considerable size reduction, cellular proliferation has not been correspondingly reduced. In fact, sensory proliferation in the graft was found to be somewhat higher than in the intact cord, and cellular proliferation in the cord as a whole is slightly above normal (table 1).

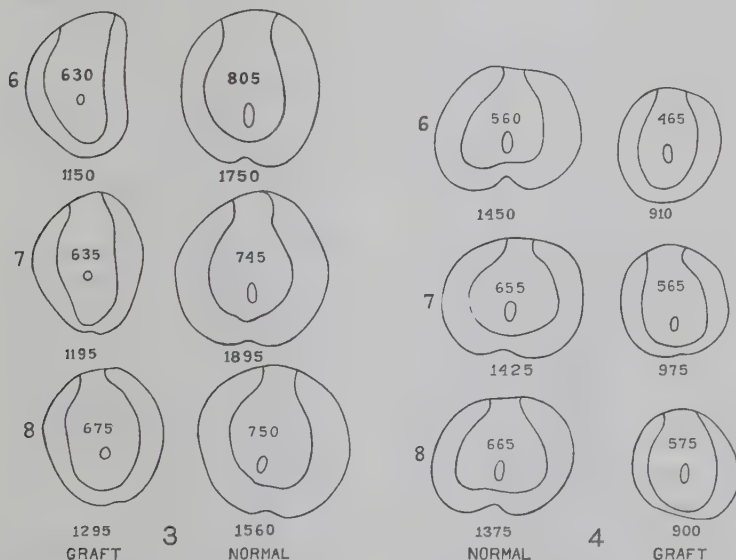


Fig. 3 Transverse sections through the sixth, seventh, and eighth segments of the normal cord and the heterotopic cord in case SCMT-16, showing cross section areas in square millimeters at $\times 100$. The heterotopic cord was grafted adjacent to the normal (v. fig. 1).

Fig. 4 Transverse sections through the sixth, seventh, and eighth segments of the normal cord, and the heterotopic cord in case SCMT-3, showing cross section areas in square millimeters at $\times 100$ (v. fig. 7).

Case SCMT-26 (52 days, 36 mm.). The host and grafted cords which are shown in figure 5 are seen to be fused at the anterior end. Caudal to this region of fusion they are separated by cartilage; more caudally, they lie within the same neural arches—a condition similar to case 16. A critical study of the size of the grafted cords in relation to the presence of separate or common neural arches for both cords has given

negative results. Whether the grafted cord is surrounded by its own vertebral arches or lies within neural arches of the host appears to have no significant effect upon size.

This case, as in the former, shows that the graft is smaller than the normal cord throughout its entire free length. Planimeter measurements of the two cords which are compared in table 1 show the markedly reduced size of the grafted cord as compared with the normal.

The cell counts from this case show that through the region of the sixth segment, the mean number of cells per section in graft and host cords are practically identical, whereas, more caudally, the counts in the graft are slightly above normal (table 1).

Case SCMT-17 (54 days, 33 mm.). Other than for an excessive growth of the grafted cord at the anterior end where it fused with the normal cord, it was smaller than the normal cord throughout its entire length and the relative sizes compare favorably with the two previous cases described (table 1, area ratios). The size of the gray area in relation to the cord as a whole is slightly greater than in the normal cord. The cell counts show that the mean number of cells per section through the sixth and seventh nerve levels of the graft are very similar to those of the normal cord, but at the eighth nerve level the count is higher in the graft than in the normal (table 1).

Case SCMT-3 (57 days, 36 mm.). In this case the grafted cord had no connection with the normal cord and the two were enclosed within separate arches throughout almost the entire course. Examination of figure 6 shows that the grafted cord is considerably smaller than the normal. Comparisons of homologous cross section areas through both grafted cords (fig. 4) are similar to case 17. The gray area through the grafted cord is seen also to be greater in relation to the cord as a whole than in the normal cord (fig. 4).

Other than for the eighth segment, the mean number of cells per section was found to be slightly lower in the graft than in the normal cord (table 1).

In the remaining cases of this series (1, 23, 4) the results agree in the main with those of the above described cases. The grafted cords are considerably smaller than the normal as estimated by reconstructions and planimeter measurements of cross section areas (table 1). The cell counts show that

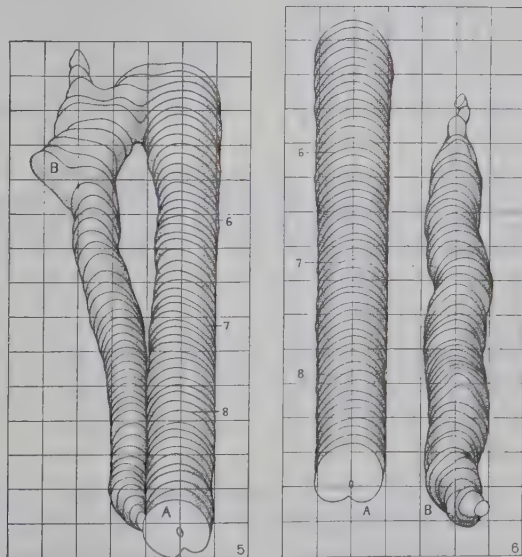


Fig. 5 Reconstruction of normal cord (A) and of heterotopic cord (B) in case SCMT-26. The heterotopic cord was grafted adjacent to the normal (fig. 1). $\times 25$.

Fig. 6 Reconstruction of normal cord (A) and of grafted cord (B) in case SCMT-3. The heterotopic cord was grafted adjacent to the normal. $\times 25$.

the mean number of cells per section in the graft may fall slightly below the normal or slightly above.

The general results of this series of experiments are shown graphically in figures 7, 8, and 9, where the average for six individual cases are given in the form of curves.

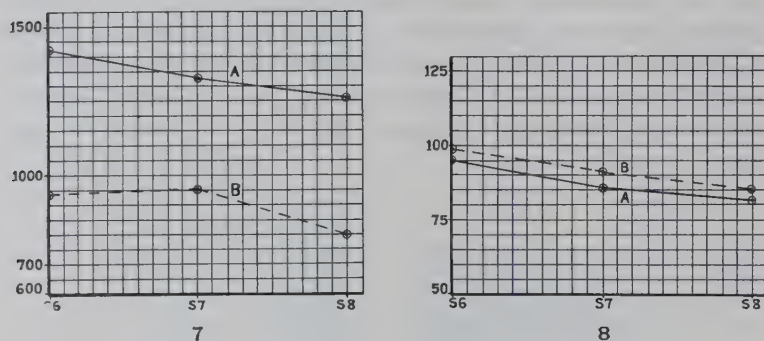


Fig. 7 Graph showing average cross section areas in square millimeters (ordinates) of transverse sections (magnified $\times 100$) through the sixth, seventh, and eighth segments (S6, S7, S8) of the normal cord (A) and the heterotopic cord (B). The heterotopic cord was grafted adjacent to the normal so that no tissue intervened (fig. 1). Curves are compiled from averages based upon six individual cases.

Fig. 8 Graph showing mean number of cells per section (ordinates) through the sixth, seventh, and eighth segments (abscissae) of the normal cord (A) and the grafted cord (B) in series SCMT. The heterotopic cord was grafted adjacent to the normal (fig. 1). Curves are compiled from averages based upon six individual cases.

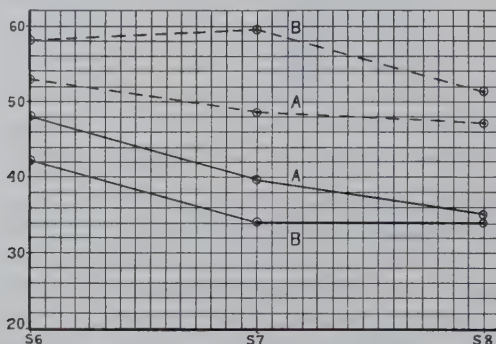


Fig. 9 Graph comparing extent of sensory (broken lines) and motor (solid lines) proliferation through the sixth, seventh, and eighth segments (abscissae) of the normal cord (A) and the grafted cord (B) in series SCMT (v. fig. 1). Ordinates are based upon averages of the mean number of cells per section at the various nerve levels from six individual cases (compare fig. 8). The heterotopic cord was grafted adjacent to the normal (fig. 1).

SERIES TrCM

In this series of experiments the sixth, seventh, and eighth segments along with the mesial portions of the adjacent somites from a donor embryo were grafted lateral to the corresponding somites in a host embryo (fig. 2), so that the conditions duplicate those in one series of experiments by Severinghaus ('30). Most of these experiments were done in stage ± 28 , although several were done in stage 23 before the mesoderm had segmented.

Whereas twenty-five cases are available, only seven are included in this study.

Case TrCMPU-6 (42 days, 36 mm.). This experiment was carried out in stage 23 before the mesoderm had segmented. Examinations of the sections showed that the grafted segments were considerably larger than the corresponding segments of the host. This is revealed in figure 13 and table 1. In fact, the increased size over the normal cord was nearly 50 per cent. The gray areas when compared with the cord as a whole are relatively larger in the grafted than in the normal cord. In this respect the results resemble those of the former series.

A study of cellular development shows that the proliferation of cells in the grafted segments is greatly in excess of the homologous intact segments. In fact, throughout the entire grafted cord, the mean number of cells per section is more than twice as high as in the homologous sections from the normal cord (table 1). This increased proliferation is especially high in the dorsal (sensory) regions, but appreciably also in the ventral (motor) regions (fig. 12).

Case TrCM-15 (53 days, 40 mm.). Reconstructions of the two cords showed again that the grafted cord was larger than the normal. This is seen also by a comparison of the cross section areas (fig. 15 and table 1). Cellular proliferation is greatly in excess of the normal (table 1) and, like the case described previously, it is particularly high in the dorsal (sensory) regions (fig. 12).

Case TrCM-14 (46 days, 31 mm.). Reconstructions of the two cords (fig. 14) show the exaggerated size of the graft as compared with the normal. An index of the size increase as estimated by planimeter readings of selected cross section areas is given in table 1.

Cellular proliferation through the grafted segments shows again an increase ranging from 40 to 100 per cent (table 1). This increase is largely in the sensory regions, although motor hyperplasia is also measurable.

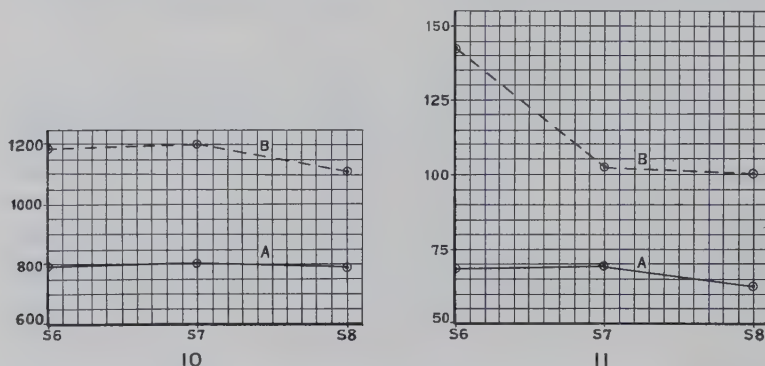


Fig. 10 Graph showing average cross section areas in square millimeters (ordinates) of transverse sections ($\times 100$) through the sixth, seventh, and eighth segments (abscissae) of the normal cord (A) and the grafted cord (B). The heterotopic cord was grafted into the lateral somitic region of the host (fig. 2). Curves compiled from averages based upon seven individual cases (compare fig. 7).

Fig. 11 Graph showing mean number of cells per section (ordinates) through the sixth, seventh, and eighth segments (abscissae) of the normal cord (A) and the grafted cord (B) in series TrCM. The heterotopic cord was grafted into the lateral somitic region of the host (fig. 2). Curves are compiled from averages based upon seven individual cases (compare fig. 8).

The remaining cases of this series show conditions similar to those just described. All of the grafted cords exhibit marked size increases as compared with the normal. Cellular proliferation is strikingly high in the dorsal regions and to a less extent in the motor regions.

The general results of this series are shown graphically in figures 10, 11, and 12 which are made up from averages of the cases listed in the table.

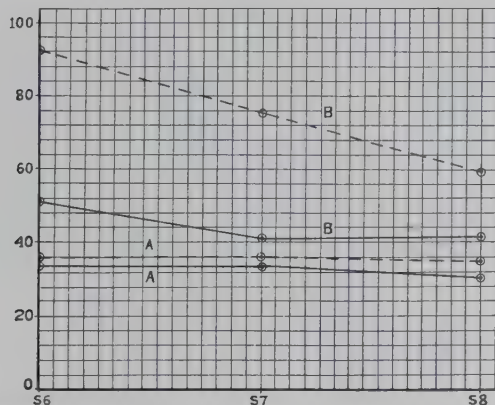
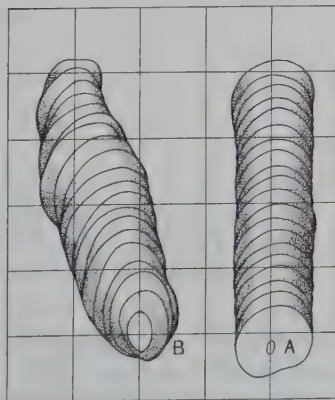
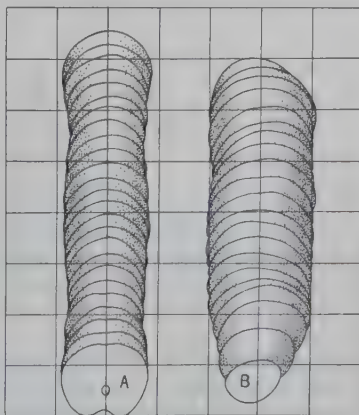


Fig. 12 Graph comparing extent of sensory (broken lines) and motor (solid lines) proliferation through the sixth, seventh, and eighth segments (abscissae) of the normal cord (A) and the grafted cord (B) in series SCM. Ordinates are based upon averages of the mean number of cells per section from seven individual cases (compare fig. 9). Heterotopic cord was grafted into the lateral somitic region of the host (fig. 2).



13



14

Fig. 13 Reconstruction of the normal cord (A) and heterotopic cord (B) in case TrSCMPU-6. The heterotopic cord was grafted into the lateral somitic region of the host (fig. 2). $\times 40$.

Fig. 14 Reconstruction of normal cord (A) and grafted cord (B) in case TrCM-14. The heterotopic cord was grafted into the lateral somitic region of the host (fig. 2). $\times 40$.

DISCUSSION

The results of the experiments described in this paper when studied in relation to those obtained by Severinghaus ('30) demonstrate clearly that heterotopic spinal cords show a striking difference in growth and cellular proliferation according as to whether they are situated adjacent to or more distant from the normal cord. Cord segments grafted adjacent to the normal suffer a size reduction ranging from

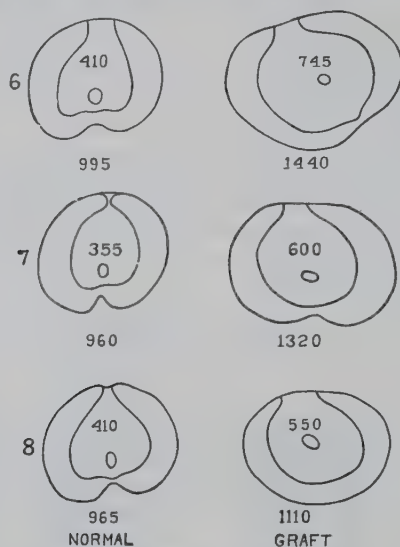


Fig. 15 Transverse sections through sixth, seventh, and eighth segments of normal cord and grafted cord in case TrCM-15, showing cross section areas in square millimeters at $\times 100$. The heterotopic cord was grafted into the lateral somitic region of the host (fig. 2). $\times 50$.

20 to 50 per cent. It was thought at first that this reduction might be due to restraining influences associated with projection tracts arising from the normal cord and invading the graft, since in all of the earlier cases examined, the two cords were fused at some point. Cases were obtained later, however, in which the grafted cord, with similar size reductions had no connection with the intact cord (fig. 6). It became obvious, therefore, that the markedly reduced size of grafted

cords lying adjacent to the normal could in no way be referred to any influences associated with projection fiber invasion. Since the cellular proliferation in these grafted cords is slightly higher than normal in spite of marked size reduction, it is clear that, whatever the nature of the inhibitory influence is, it affects general growth much more than it does cellular content. One might assume that growth in these cords is mechanically held in check by the surrounding tissues, yet there is little if any positive evidence for this. Grafted cords adjacent to the normal suffer a similar size reduction regardless as to whether they lie within the same neural arches as the normal or whether they possess their own arches. The presence of much, little, or no surrounding cartilage seems to have no definite effect upon growth in size. A search for an influence on the part of the notochord resulted negatively. Whereas all of the grafted notochords are much smaller than the normal, it is found that they are likewise subnormal in size when associated with those cords grafted lateral to the somites where growth in the grafted segments is greatly in excess of the normal.

If the size of grafted cords were mechanically held in check by surrounding cartilage, one might expect those grafted lateral to the somites to be no larger than those lying adjacent to the normal cord, for, under the former conditions, the grafted cord is, in all cases, surrounded by cartilaginous arches—yet it is seen from the experiments that cords lying lateral to the somites grow much larger than the host cord, whereas those adjacent to the host cord are smaller than normal.

Since cellular proliferation is also much greater in the laterally placed cords than in the adjacently situated ones, it is clear that there is some sort of inhibitory influence which lessens as the distance between normal and grafted cord increases.

The fact that spinal cords, when grafted adjacent to the normal, grow to smaller size without any connection of substance, does not lessen the value of the suggestion by Sever-

inghaus that, normally, certain invading projection fibers within the central nervous system may call forth differentiation and thus put an end to proliferation. It is apparent, therefore, that cord segments, when grafted beyond the confines of the normal environment of the central nervous system, are free to grow to large size and to proliferate excessively because they are not subjected to those influences which normally terminate proliferation and bring about differentiation. However, if the cord segments are grafted adjacent to the normal, their growth and differentiation may be restrained also by influences other than those associated with projection fiber invasion. These influences appear to be referable to some condition associated with the nearness of two like structures. Evidence of similar inhibitory influences has been observed in connection with the growth of one of two limb rudiments when developing adjacent to each other (Detwiler, '20 b).

There seems to be no doubt from the results of Severinghaus and their support in the present experiments that the spinal cord has a much higher potential for growth and cellular proliferation than is normally expressed.

SUMMARY

1. When the sixth, seventh, and eighth spinal cord segments of *Amblystoma* embryos are grafted adjacent to the corresponding segments of the host cord without the presence of any intervening tissue, they undergo a size reduction ranging from 20 to 50 per cent of the normal. In spite of this marked size reduction, slight cellular hyperplasia may result. The mean number of cells per section in the grafted segments may be slightly less, equal to, or slightly higher than in the normal control cord (table 1). When hyperplasias occur, they are confined to the dorsal (sensory) region of the cord. The ventral (motor) regions tend to suffer a slight hypoplasia.

2. When units of cord involving the same three segments are grafted with adjacent mesoderm into the lateral somitic region of a host embryo, they become greatly enlarged over

the normal control cord. This enlargement may vary from 20 to 100 per cent of the normal. Cellular proliferation in the grafted segments is extremely high as compared with the normal cord and may reach an increase of from 200 to 300 per cent (table 1). This hyperplasia is much greater in the dorsal (sensory) regions than in the ventral (motor). These observations upon growth and cellular development in laterally grafted cords are in general agreement with those obtained previously by Severinghaus ('30).

3. In heterotopically grafted units of cord, the size of the gray (cellular) region in relation to the size of the cord as a whole is greater than in the normal cord regardless as to whether the graft is adjacent to the normal or in a more distant situation.

4. The correspondence between size and cellular increases is much greater in heterotopic cords lying in the lateral somitic region than in cords lying adjacent to the normal. In the latter position, the gray areas in spite of size reduction may show appreciable cellular hyperplasias.

5. The restricted growth and cellular proliferation in heterotopic cords lying adjacent to the normal as compared with those lying in the lateral somitic region, argues for the presence of a growth inhibitory influence associated with the presence of the normal cord. Within limits, this influence diminishes as the distance away from the normal cord is increased. There is no proof that the restricted growth and cellular development in the heterotopic cords lying adjacent to the normal as compared with those more distantly located is caused by any greater mechanical restrictions of the environment.

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BOOK REVIEW

SEX AND INTERNAL SECRETIONS: A SURVEY OF RECENT RESEARCH. Written by twenty-one contributors, edited by EDGAR ALLEN. Octavo, xxii + 951 pp. Baltimore, The Williams and Wilkins Company, 1932.

The past three decades have witnessed a development of our knowledge of the biology of reproduction such as was never before achieved in a like span of years except perhaps in the period 1651-1685, which saw the publications of Harvey, de Graaf, and Leeuwenhoek. In this new advance American investigators have taken a remarkably large part, beginning with McClung's formulation of the sex chromosome theory (1902), Bennett Allen's investigations of gonad embryology (1904 ff), and the work of Leo Loeb (including the deciduoma experiment of 1907) and stimulated by two great advances in 1916-1917, namely Lillie's analysis of the free-martin problem, and Stockard and Papanicolaou's description of the vaginal cycle in rodents. In recent years the resources of the universities have been augmented by grants of the Committee on Sex Research of the National Research Council, and the number of workers has been recruited until work in this field has become one of our most important branches of science.

To mark the first decennial of its part in this work, the Committee on Sex Research has sponsored the publication of 'Sex and Internal Secretions' as a review of recent progress, especially of those phases of the subject upon which the Committee's interest has been chiefly concentrated. Frankly intended as a sort of stock-taking by the experts who have been leading the work, this admirable volume is designed to serve also as a handbook for present and prospective investigators and for persons with biological interests who wish to keep up with the subject. Prepared by twenty-one writers, whose combined texts amount to over nine hundred pages, the book is indeed almost as encyclopedic as it is authoritative. Here is no light diet for the casual, but solid fare for professional workers.

Except for the general plan, the hand of the Editor, Edgar Allen, seems to have been laid very lightly upon the contributors. They have not only been given full responsibility for their expressions of opinion, but have been permitted their own whims of terminology and of diction; those who like 'Amblystoma' for example may use the term

and those who prefer may dispense with the 'l'; likewise with 'oestrum' and 'oestrus.' Authors may invent totally new words (e.g. 'aviatic' on page 212). They may call the same hormone by three or four different names (but more later on that topic). Such editorial liberality preserves the freshness of individual style and has the further admirable result that it permits the expression of quite contradictory opinions, as for example those of Bridges (page 74) and of Witschi (page 176) on the subject of the unity of the gene.

The first five chapters, dealing with genetic and embryological aspects of the problem, make a kind of symphony, with its themes stated by Professor Lillie in his introduction, and developed in turn by Danforth in an excellent chapter on Genic and Endocrine Factors in Sex, Bridges on Genetics of Sex in *Drosophila*, Willier on Embryology, and Witschi on Deviations, Inversion, and Parabiosis. The five chapters together give an instructive and surprisingly harmonious exposition of the question of genic versus gonadal determination of sex, all the authors accepting a view in which the sex genes act simply to direct the undifferentiated gonad toward maleness or femaleness, the gonad then inducing the germ cells to become either oocytes or spermatocytes, and at the same time controlling the accessory sex characters by hormonal action. An important gap in the theory relates to the control of the primitive and least modifiable characters, especially the external genitalia, which show some response to hormonal action, but are, on the whole, relatively refractory. Because of their very early development and strong determination, they appear to be under the control of genetic more than of hormonal factors. In one of the most interesting passages in the whole book, Bridges goes beyond his immediate subject to outline a general theory of gene-action in terms of ordinary chemical concepts, which to this reviewer at least makes plausible reading.

Chapter VI, Metabolism and Sex, by Riddle, is given over to the author's metabolic theory of sex. Dr. Riddle for years stood practically alone (in the United States) in questioning the more rigid sex-chromosome theory. For this reason his views will always obtain a hearing, and it will be admitted that oxidation-reduction mechanisms must play some part in this as in other activities of animal tissue. As stated in this chapter, however, the question is not precisely defined, for Riddle begins on page 246 with the statement that the specific sex differential of the oxidation rate is the primary and really decisive element, beyond chromosomes and genes; while he concludes (page 273) by assuming that the chromosomes or genes exercise their influence on developing sexuality by establishing higher or lower oxidation rates. To the support of ideas so difficult to test by direct experiment, Riddle brings many observations from the literature, some of

which he is himself too critical to apply outright, and therefore the chapter is laden with 'ifs' and other expressions of caution which greatly weaken the argument.

Moore deals, in workmanlike fashion, with the biology of the testis, and in particular its hormone or hormones. With regard to the actively controverted question as to the relative endocrine importance of spermatogenic epithelium and interstitial cells, Moore weighs the evidence to indicate that in lower vertebrates the germinal epithelium may be concerned in hormone production, but that in birds and mammals the interstitial tissue can function without cells of the germinal line. Koch describes clearly what is known of the chemistry of the testis hormone ('comb-growth' hormone), its general actions, and the technique and difficulties of assay.

The Editor's chapter on the ovarian follicular hormone is thorough and instructive. All the known animal reactions are clearly discussed, and the author's pioneer experiments on the endocrine control of the menstrual cycle in monkeys are fully reviewed in the light of new information about the pituitary and corpus luteum. In this and in still less settled fields which he treats in conclusion, such as the apparently special situation with respect to oestrin in the primate placenta, the interrelations of the ovaries and other endocrine glands, and evolutionary trends in ovarian function, Allen's article is full of useful conjecture and explanation. Doisy gives a lucid account of the biochemistry of oestrin in general, of the crystalline substances obtained from human pregnancy urine (named by him 'Theelin' and 'Theelol'), and of the rapidly successive stages by which in 1930-31 in four laboratories in the United States, Germany, England, and Holland the molecular formulae of these substances were obtained. One would have welcomed an opinion as to the apparently similar derivative of the placenta made by Collip under the name 'Emmenin', which has caused some stir in clinical circles, especially in Canada. The matter of bio-assay seems too briefly discussed, but one gets a fairly complete account of this subject by reading Allen's section II, 6 (page 406) in connection with Doisy. There is on pages 484-485 a valuable table of the distribution of oestrin in various plant and animal tissues.

Hisaw, discussing in Chapter XI the physiology of the corpus luteum, gives an accurate and complete account of the subject; the same is true of Turner's discussion of the mammary glands, which deals clearly with the confused situation recently prevailing in the field. Domm, Gustavson, and Juhn, in the first part of their chapter on plumage tests in birds, round up very intelligibly the present knowledge of sex relations of feathering, and in the latter part summarize their experiments which are giving clues as to the dynamics of hormone control of sex characters as found in the feathers.

The chapter on Ovulation and Transport and Viability of the Ova and Sperm will no doubt prove one of the most interesting in the book to the general reader, for Hartman has summarized with skill and clarity a mass of information not heretofore assembled in one place, which is highly important for human reproduction. P. E. Smith, Engle, and Severinghaus have divided up the complex subject of the hypophysis in relation to reproduction. As a result the field is very thoroughly covered, except on the biochemical side. Work on the hypophysis is now in a stage of reconnoitering and cross-checking after the very great advance of recent years, in which Smith and his colleagues have taken a major part. It is perhaps inevitable that the three sections convey a sense of this unsettlement so strongly that a reader new to the field might not realize the immense achievement underlying it. A page or two of general summary of the whole subject, with emphasis on the larger established facts, would help much.

Stone describes the work, now in its infancy, on the measurement of sexual drive. It is a good thing to have the technique of experiment and control developed by the psychologists set down for the benefit of physiologists at this early stage of cooperation between the two sciences. J. P. Pratt, the only medical man in the corps of authors, undertakes the difficult task of discussing endocrine disorders of sex in man. That a brilliant review of positive achievement can not now be written goes without saying. In the reviewer's opinion such caution as displayed by Pratt is necessary to sound advance in the future.

Obvious gaps in the book are few. A good chapter on the oestrous cycles of various species would aid in completeness, but the subject is well treated elsewhere (by F. H. A. Marshall) and there is after all no American specialist. The vaginal cycle of rodents, so important for many of the researches here discussed, is given only incidentally; perhaps it has become axiomatic. One would like to have a full review of the important question of the postnatal formation of ova. Menstruation and the human cycle are given only scattered and incomplete discussion.

This reviewer feels it a duty to deplore publicly the attempt of several writers to make a general term of the word 'Theelin', introduced by Doisy to indicate a crystalline substance of formula $C_{18}H_{22}O_2$, derived from human pregnancy urine; it is protected by a university-held patent and is licensed to one particular manufacturer. That the use of this term to signify the follicular or female sex hormone in general leads to ambiguity even among the Olympians, will be seen by comparison of Allen's remarks on page 400, l. 20 ("theelin has been demonstrated in the follicles . . .") with Doisy's on page

486, 1. 10 of footnote ("there is no absolute proof that the follicular hormone is either theelin or theelol"). The word 'oestrin' has been gaining general acceptance in this meaning; it seems a pity to insist upon another and ambiguous name.

The publishers have contributed much to make the book convenient, by the use of legible type and a light-weight hard uncoated paper on which the numerous illustrations have come out well. The typography is not quite *sans tache*, forty-six misprints (all trivial) having been incidentally noted by the reviewer. The bibliographies are full and clearly arranged, with complete titles. There are ample indexes of authors and subjects.

In a book which will be the daily guide of many graduate students and other newcomers to the field, it is gratifying to note the general spirit of enthusiasm, fairness, and good-will. To the Editor and the collaborators of this notably successful and useful book will go the congratulations and thanks of all their fellow-workers.

GEORGE W. CORNER.

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

LABORATORY ATLAS OF THE 13-MM. PIG EMBRYO (Prefaced by Younger Stages of the Chick Embryo), by Edward A. Boyden. 1933. Size, $8\frac{1}{2} \times 11$ inches. Pages iv + 100. 66 illustrations. Bound in waterproof buckram. Price, \$2.00. The Wistar Institute of Anatomy and Biology, Philadelphia.

This guide to the study of vertebrate organogenesis gives the student detailed first-hand knowledge of the development of mammalian organs and systems. It is illustrated with reproductions of wax models and graphic reconstructions, and drawings of representative sections. The very early stages are studied in the chick. Organogenesis is studied in the 13-mm. pig. Pictures of forty sections, drawn under the Edinger projection apparatus, save the time and labor usually expended of necessity by the student in drawing them for himself. The drawings as printed in the book are mechanically perfect and are ready to be labeled as corresponding sections are studied under the microscope. The book is printed on a heavy ledger paper and is interleaved for the student's notes and supplementary drawings. The durable waterproof binding is well adapted to use in the laboratory.

SURGICAL ANATOMY, by C. Latimer Callander, with a foreword by Dean Lewis. 1933. Size, $8 \times 10\frac{1}{4}$ inches. 1115 pages. 1380 illustrations, some of which are in colors. Index. Buckram. Price, \$12.50. W. B. Saunders Company, Philadelphia.

Recognizing that accurate anatomic knowledge is the basis of surgical technique, the author has presented his facts in terms of their clinical importance, describing topographically the anatomic surgical approaches, the paths of extension of pathological processes, and the commoner operations. Doctor Lewis, in the foreword, outlines the most effective method of teaching gross anatomy. "In order to obtain the best results, as far as the clinic is concerned, regional and topographical anatomy must be emphasized. The systems should not be regarded as entities. When the breast is studied anatomically, all the structures entering into its formation should be visualized in their proper relationship. Acini ducts, for example, should be considered in their relationship to the lymphatics; both the normal accessory lymphatic channels in relation to their central connections and to the lymph nodes. Muscles and bones should be considered together so that the student may determine while the subject is fresh in his mind the relation of muscles to the displacement of fragments in fractures. The strengthening bands in the capsules of joints may be advantageously studied in their relation to different types of inflammation. To give the name 'monkey hand' to the deformity caused by combined division of the ulnar and median nerve no longer

NEW BOOKS AND PUBLICATIONS

satisfies, for the student should know the muscles affected, the action of each, and the relation of the antagonistic groups causing the deformity. Such an approach stimulates thinking and vitalizes a subject which to many may seem dead. Visualization rather than memory alone should be cultivated." This book satisfactorily approaches this ideal. Anatomy and its surgical application are considered in close sequence, systems and organs are studied in relation to each other, and the many excellent illustrations encourage visualization.

A LABORATORY MANUAL OF NEURO-ANATOMY. Part II. Stereographic Plates, by C. L. Davis and H. S. Rubenstein. 1933. Size, $7 \times 9\frac{1}{2}$ inches. Price, \$3.00. William Wood & Co., Baltimore.

A series of stereograms worked out at the University of Maryland for use in teaching neuro-anatomy. It consists of thirty-one cards fitted into a case and showing thirty different views of the human brain. The stereograms are photographic reproductions of original specimens and models. Each card bears, in addition to the stereograms, a line drawing clearly lettered, emphasizing and identifying important structures. By means of these illustrations the pupil is able at any time to study material usually available only in the laboratory. The views have been selected to show the arrangement and relation of the parts of the brain, and should prove a valuable aid in learning a difficult subject.